I. Application for a Permit for Scientific Research to enhance the survival or recovery of a stock under the Marine Mammal Protection Act and the Endangered Species Act.

I. Date of application: 07 March 2002

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III. Applicants and Personnel

A. Applicant:

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Co-Investigators:

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Dr. Shannon Atkinson
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Dr. Natalie Noll
Dr. Alexander Burdin
Mr. John Maniscalco
Mr. Jason Waite
Ms. Kendall Mashburn
Dr. Markus Horning
Dr. Russell Andrews
Ms. Daniela Maldini
Dr. Lorrie Rea
Mr. Bob Hicks
Dr. Lisa Mazzaro

B. Qualifications/experience of PI and CI(s)*

1. Don Calkins is the Steller sea lion Program Manager at the Alaska SeaLife Center, and a member of the Steller sea lion Recovery Team . He has been a prominent researcher in pinniped biology and has worked in the field with Steller sea lions for almost three decades. He has also worked with many other marine mammals, including harbor seals, sea otters and belugas. Primary tasks will be the supervision all of the included Tasks, as well as principal investigator with the Remote Video Monitoring, Free-ranging juvenile health assessments, Transient juvenile program and Development of a floating platform trap method.

2. Jo-Ann Mellish, PhD., is an Assistant Research Professor at the University of Alaska Fairbanks and a Scientist at the Alaska SeaLife Center. She has worked with pinnipeds in captivity and in the field for seven years, including species such as grey seals, harbor seals, hooded seals, Steller sea lions and northern fur seals. Primary responsibilities will be to help coordinate the multiple Tasks outlined, as well as being an investigator with the juvenile health assessment, life history transmitter and 3D photogrammetry assessment tasks.

3. Shannon Atkinson, Ph.D., is a Professor at University of Alaska Fairbanks, and serves as the Science Director at the Alaska SeaLife Center. She is also a member of the Steller sea lion Recovery Team. She has been involved in marine mammal research for over ten years, including work with Hawaiian monks seals and Steller sea lions. Primary responsibilities will involve supervision of all included Tasks, as well as investigator in the juvenile health assessments, transient juvenile captive program and reproductive failure tasks.

4. Pam Tuomi, D.V.M., is the senior veterinarian for the Alaska SeaLife Center and a consultant for the US Fish and Wildlife Service, National Marine Fisheries Service and the Canadian Wildlife Service. She has been involved in research with marine mammals for over a decade, including Steller sea lions, sea otters and walrus. She will be primarily involved with the juvenile health assessments and all aspects of the juvenile transient captivity program.

5. Natalie Noll, D.V.M., is the rehabilitation manager for the Alaska SeaLife Center. She will assist in any tasks that require veterinary supervision.

6. Alexander Burdin, Ph.D., is a Senior Scientist for the Kamchatka Institute of Ecology and Nature Management, and a visiting scientist at the Alaska SeaLife Center. Primary responsibilities will include the remote monitoring task.

7. John Maniscalco is a Research Associate at the Alaska SeaLife Center. He has participated in marine mammal research, including Steller sea lions and harbor seals, for ten years. Primary responsibilities will include the remote monitoring task.

8. Jason Waite is a Research Associate at the Alaska SeaLife Center, with ten years of field and experience with the Center and the Alaska Department of Fish and Game. Primary responsibilities will be participation in the remote monitoring station, general juvenile health assessment (capture) and the transient juvenile captive program (remote monitoring with external tracking devices).

9. Kendall Mashburn is the Science Program Coordinator at the Alaska SeaLife Center. She has over 15 years of experience with a variety species in veterinary and captive environments. Primary responsibility will be involvement in the collection and analysis of samples for general health assessments (endocrinology), the transient juvenile program (stress indicators, endocrinology) and reproductive failure investigation.

10. Markus Horning, Ph.D. is an Associate Professor at Texas A&M University. He has twenty years of experience with pinnipeds and has developed biological software programs and hardware for remote monitoring and data collection. He will primarily be involved as an investigator with the general juvenile health assessments, the juvenile transient captivity program and the carcass validation studies.

11. Russell Andrews, Ph.D., is an Assistant Research Professor with the University of Alaska Fairbanks and Scientist with the Alaska SeaLife Center. He has ten years of experience in Marine Biology and has experience in the physiology of pinnipeds. He will participate in the Remote Video Monitoring task.

12. Daniela Maldini is a Research Associate at the Alaska SeaLife Center. She has worked in the field of marine mammalogy for ten years and a total of eighteen years of biological experience. She will primarily assist in tasks whenever required.

13. Lorrie Rea, Ph.D., is a Wildlife Biologist with the Alaska Department of Fish & Game. She has over ten years of experience working in the field and analysing data for pinnipeds. Primary responsibilities will be as an investigator with the transient juvenile captive program (fatty acid signatures).

14. Bob Hicks, J.D., serves as the dive safety officer at the Alaska SeaLife Center and will be involved in the training and leading of dive capture teams for the transient juvenile program.

15. Lisa Mazzaro, Ph.D., is a researcher at Mystic Aquarium. She has been involved in marine mammal research for over ten years, and has extensive experience with vitamin research in pinnipeds. She will be an investigator with the transient juvenile program (vitamin requirements).

Additional qualifications and experience of the PI and CI(s) are attached in **Biographical Sketches**.

IV. Description of Proposed Scientific Research

A. Summary

This permit application covers the full scope of research to be performed by the Alaska SeaLife Center (ASLC) and associated authorized collaborators on free-ranging and temporarily captive (transient) Steller sea lions. There are five comprehensive projects included in this overall program, which address current research associated with the decline of the western Steller sea lion stock. Among the major investigations are comprehensive health assessments of juveniles, implementation of the life-history transmitter program, remote imaging and monitoring of rookeries, marking and re-sight of individuals, and collection of dive behavior and location data via external tags. We will also implement a captive program at the Alaska SeaLife Center, which will involve the capture, handling and short-term captivity (≤ 3 months) of juvenile Steller sea lions (1- 4years). These juveniles will be involved in 7 studies, including assessments of general health status, the life history transmitter project, mass and body composition estimates using 3D photogrammetry, stress responses to capture, handling and captivity, vitamin requirements and fatty acid signatures. These studies will provide data on juvenile survival, population dynamics, immunology, epidemiology, endocrinology, viral serology, physiology, ontogenetic and annual body condition cycles, foraging behavior and habitat usage. The goal of the Alaska SeaLife Center is to provide and facilitate quality research that will assist in the recovery of the Steller sea lion, and accordingly these studies combined will address objective sections 1, 2, 4, 5 and 6 of the Steller sea lion Recovery Plan (1992).

B. Introduction

1. Hypothesis/Objectives: The overall objective of the free-ranging Steller sea lion research program is to aid in the investigation of the decline of the western stock and its failure to recover and to assist recovery efforts through the accumulation of essential information. Given the large number of projects included in this research program, specific objectives are outlined with each respective project description below. The field components of these projects will take place throughout the Alaskan range of the Steller sea lion. The objectives of each project are listed below:

Task 1. Remote video monitoring

1. Study the multi-year/seasonal/daily distribution, abundance, and movements of Steller sea lions on the Chiswell island rookery and other haul-out sites and rookeries in the Gulf of Alaska throughout the year
2. Describe the breeding period, determine male reproductive strategy (breeding success), and evaluate maternal investment
3. Provide sightings of branded animals for use in estimating survival of newborn pups and assist in making survival estimates of juveniles
4. Create a photo/video database of individually recognizable sea lions
5. Develop a remote satellite-linked video monitoring system
6. Establish a low impact blind/platform on Chiswell Island

This task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): conduct visual marking/tagging studies (sections 21, 211 & 443), monitor rookeries for the occurrence of marked animals (section 212), conduct studies on rookeries (section 44), determine age and sex classes of animals on shore (section 441), determine rates of pup production and mortality (sections 442, 512 & 517) and monitor status of tagged animals (section 444).

Task 2. Free-ranging juvenile health assessment

1. Determine basic hematological and clinical chemistry profiles for free-ranging juveniles
2. Determine the presence and level of infectious agents in juveniles
3. Determine the level of immuno-competence in juveniles
4. Determine the level of pollutants in juvenile tissue
5. Determine the body condition of juveniles
6. Determine the level of stress indicators in juveniles

This task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): determine if biological parameters indicate different stocks of sea lions (section 22), monitor health, condition and vital parameters (sections 4, 43, 432, 445, 46, & 47), and investigate sea lion feeding ecology (section 61, 613, 6131 & 6132).

Task 3. Transient juvenile program

1. Facilitate the implementation of multiple short-term research projects that cannot be accomplished with long-term captive or free-ranging animals to enable the validation of the Life History Transmitter project paradigm
2. Remotely track released individuals to obtain dive history and location data for comparison with non-transient animals

3. Monitor and study the overall effects of surgery on juveniles
4. Study the effects of surgery and short-term captivity on stress levels
5. Enable the validation of the use of 3D photogrammetry for the estimation of body mass and body condition
6. Establish fatty acid signature profiles for juvenile Steller sea lions for metabolic and dietary interpretation
This task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (sections 4, 43, 432, 445, 46, & 47), assess causes of mortality (section 5), investigate sea lion feeding ecology (section 6, 61, 611 613, 6131 & 6132).

3.1. Life history transmitters

1. Determine the survival rate for juvenile Steller sea lions through life history transmitter implantation
2. Determine the time of year for the greatest mortality for juvenile sea lions
3. Determine approximate locations of mortalities
4. Obtain dive behavior and dive effort from deceased animals
5. Test the effects of body condition, health, pollution and immuno-competence on survival of juvenile Steller sea lions
6. Assess the predictive power of parameters measurable in juvenile Steller sea lions for future survival

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (sections 4, 43, 432, 445, 46, & 47), assess causes of mortality (section 5), investigate sea lion feeding ecology (section 6, 61, 611 613, 6131 & 6132).

3.2. Monitoring of transient juvenile Steller sea lions with external tracking devices

1. Compare the post-release behavior of life history transmitter-implanted transient juveniles to that of non-transmitter implanted transient juveniles
2. Compare the post-release behavior of captive transient life history transmitter-implanted individuals to free-ranging life history transmitter-implanted individuals
4. Compare the post-release behavior of captive transient juveniles to records from free-ranging animals (previously collected and/or future records)

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): develop methods for non-lethal sampling (section 43), investigate sea lion feeding ecology (section 61, 613, 6131 & 6132).

3.3. Monitoring of stress responses in short-term captive Steller sea lion juveniles

1. Assess the immediate and short-term physiological response to capture and captivity
2. Assess the physiological effects of life history transmitter implant surgery

to determine what concentration ranges of glucocorticoids indicate a distressed state in juvenile Steller sea lions

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (sections 432 & 445).

3.4. Assessment of 3D photogrammetry as a tool for estimation of body mass and condition

1. Define the accuracy of body mass calculations based on relationships derived from morphometric measures
2. Assess the validity and accuracy of estimating body condition using 3D photogrammetry
3. Assess the comparability of body condition estimates using 3D photogrammetry with existing methods of measurements (i.e., morphometrics, isotope dilution, ultrasound blubber depth)
4. Determine patterns of blubber mobilization under fasting conditions (e.g., preferential mobilization of given body regions)
5. Determine trends of fatty acid mobilization under fasting conditions (e.g., preferential mobilization of selected fatty acids).

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (sections 4, 43, 432, 445, 46 & 47).

3.5. Fatty acid signatures of juvenile Steller sea lions

1. Collect a base-line fatty acid signature from juvenile Steller sea lion blubber and blood samples
2. Determine which fatty acids are mobilized from the blubber store during a fast (in conjunction with **Task 3.4**)
3. Monitor fatty acid signatures of captive, transient juveniles fed a homogenized diet

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (section 432 & 445, 46, 47), and investigate sea lion feeding ecology (section 611).

3.6. Vitamin requirements of juvenile Steller sea lions

1. Establish the status of free-ranging Steller sea lions with respect to the fat-soluble vitamins A (retinol) and E (tocopherol)
2. Determine the metabolic requirements of Steller sea lions for vitamins A and E by relating intake to blood levels in captive specimens.

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (section 432, 445, 46 & 47).

3.7. Metal toxicity in Steller sea lion cell lines

1. Establish continuous cell lines to determine the toxicity of metals to Steller sea lions

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (section 4, 43 & 46).

<u>Task 4. Opportunistic utilization of Steller sea lion carcass/tissue sources</u>

1. Opportunistically utilize carcasses and/or portions thereof to examine parameters relating to the overall Alaska SeaLife Center Steller sea lion research plan and the Steller sea lion Recovery Plan (1992).

4.1. Carcass validation of life history transmitter project

1. Directly assess the suitability of life history transmitter technology in Task 3.1.

This task is necessary to allow for the successful implementation of Task 3.1, which addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (sections 4, 43, 432, 445, 46, & 47), assess causes of mortality (section 5), investigate sea lion feeding ecology (section 6, 61, 611 613, 6131 & 6132).

4.2. Assessment of reproductive failure in Steller sea lions

1. Document known cases of abortion and neonatal death in Steller sea lions
2. Examine the role of bacterial infections as a cause for abortion in the Steller sea lion
3. Examine the basic structure and function of Steller sea lion placentas, including enzymology, contaminant load, immunoglobins and endocrinology
4. Examine aborted fetuses and deceased newborns for enzymology, contaminant load and endocrinology

This task meets the following recommendations/objectives of the Steller sea lion Recovery Plan: monitor health, condition and vital parameters (section 4, 41 & 46).

4.3. Development of a cell line for the determination of metal toxicity in the Steller sea lion

1. Establish continuous cell lines to determine the toxicity of metals to Steller sea lions

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (section 4, 43 & 46).

Task 5. Development of a floating platform trap method for the capture of Steller sea lions

1. Develop floating platform traps for use with Steller sea lions in Alaska
2. Expand the capability for capture of larger pups, juveniles, sub-adult and adult Steller sea lions
3. Minimize the need for immobilizing drugs for sampling of larger age classes
4. Expand the proportion of the population monitored

This task meets the following recommendations/objectives of the Steller sea lion Recovery Plan: monitor health, condition and vital parameters (section 4, 43, 431, 443 & 445).

2. Status of the Affected Stock:

a. *Species description*:

Eumetopias jubatus, Steller sea lion (Western US stock). No other protected species are anticipated to be taken incidentally during the course of the research activities described in this application.

b. *Life History and Population Status*:

The Steller sea lion is the largest otariid, weighing approximately 23 kg at birth, and increasing to approximately 545 kg and 260 kg respectively, for adult males and females. During the months of May through July, Steller sea lions are found on rookery sites to give birth, to begin lactation, and to breed. Females characteristically give birth to their first pup around the age of four, whereas males are not typically large enough to hold a territory and are therefore not likely to breed until at least 8 years of age. Pups are often weaned after one year, although suckling pups as old as 3 years have been observed.

Steller sea lions are found throughout the North Pacific Ocean, Bering Sea and northwest coasts of the US and Canada, reaching south to central California. Two distinct populations have been identified within the US, the eastern and western stock, with the division at Cape Suckling,

Alaska (144ºW). Population counts of the western stock during the 1970's exceeded 109,000 individuals; however, the most recent counts in 1998 totalled 20,201 individuals, with a total population estimate of 39,031 (NMFS Stock Assessment 2000). Therefore, the western US stock of Steller sea lions is currently listed as 'endangered' under the Endangered Species Act, and 'depleted' under the Marine Mammal Protection Act. Definitive causes for the decline are unknown, although interactions and /or competition with fisheries, predation, disease and environmental change are suggested sources (Steller sea lion Recovery Plan 1992). The smaller eastern stock of the Steller sea lion, however, has not displayed an overall decrease, but instead an overall increase. Counts of non-pups and juveniles totalled 21,864 in 1998, compared to 18,754 in 1992, and 15,214 in 1982 (NMFS Stock Assessment 2000). The current total minimum estimate is 30,403. The Eastern stock of the Steller sea lion is currently listed as 'threatened' under the Endangered Species Act, and 'depleted' under the Marine Mammal Protection Act.

3. Literature Review:

Each project to be covered under this permit application will be addressed, and denoted by Task.

Task 1. Remote Video Monitoring

Direct observations on haul outs and rookeries have long been essential for detailed investigations of Steller sea lions. Instantaneous observations and photographs are used in counting sea lions; visits of a few minutes to a few hours are made for tag and brand sightings and daily observations over long periods of time have been used for both of these as well as a large variety of behavioral and observational studies with naturally marked individuals. Steller sea lion rookeries are usually located on remote rocks and small islands that are hard to access in order to conduct long-term observations. Access to rookeries for research purposes is commonly limited by weather conditions or disturbance of the animals on approach. To have a better understanding of Steller sea lion life history, it is important to know how often sea lions visit different rookeries or haul-out sites, how long they stay in each location, and what the daily and seasonal abundance of different sexes and age classes are at these rookeries and haul-out sites. Sea lions will periodically occupy all of the available areas of a rookery or haul-out. However, it will be difficult to access and view animals. In some instances access is not possible and observations must be conducted by boat. While conducting direct, continuous, long-term observations of sea lions on different types of rookeries and haul-out sites is critical for the assessment and management of sea lion populations, collection of such data is difficult at many locations because it is impossible for researchers to remain on many sites or access may be restricted to helicopters.

In 1998, the Alaska SeaLife Center began a remote video project on Chiswell Island near the entrance to Resurrection Bay to count sea lions and conduct behavioral studies. This work was conducted under a Fish and Wildlife Service permit to the ASLC (#01-015) and Office of Protected Resources permit issued to the National Marine Mammal Laboratory (# 782-1447, 782-1532). Video cameras with associated support equipment and a microwave transmitter were installed on Chiswell Island near the Steller sea lion rookery. Most of the installation was accomplished when sea lions were not present in late winter and early spring. Maintenance was approximately once a month following installation during the first two years, but much less in the current year of study. Disturbance to sea lions was minimal. However, access was often gained by helicopter which caused some sea lions to enter the water. Examples of this

disturbance for maintenance purposes are shown in **Figure 1**. Microwave transmissions through a single relay system bring a live controllable video image into the ASLC. This image is provided by up to 6 different cameras, each of which are controllable for pan, tilt, and zoom. In this manner nearly the entire rookery area can be viewed through the remotely controlled video cameras. The application of this new technology has provided a new insight into the biology of Steller sea lions during their reproductive season.

Remote monitoring of the Chiswell Island rookery has been a successful demonstration project. It provides real-time and full-time daylight hours coverage of a Steller sea lion rookery that would otherwise not be possible. Field camps to provide similar coverage would be more costly and would result in additional disturbance to the rookery, especially during set-up and teardown of the camps. Although census data would be available from a field camp, full-time images would not. After demonstrating that this system would work, remotely controlled video cameras with transmitters will be deployed at two additional nearby locations. These locations will be determined based on patterns of animal presence, but may include the haul out at St. Mary's Bay on Rugged Island and Cape Resurrection on the Resurrection Peninsula. This will provide additional information on where sea lions may go when they are not present on Chiswell Island. The best results of any kind of monitoring of natural populations can be obtained using individually recognizable animals, so NMFS branded and tagged sea lion pups at rookeries in the Aleutian Islands and Gulf of Alaska including Chiswell Island. This has provided permanently marked sea lions that allow the video monitoring effort to contribute to assessing survival and return rates of sea lions to their natal habitat.

In order to facilitate operations on Chiswell Island for marking animals and occasionally sampling animals, a low impact, portable camouflaged blind/ platform will be constructed at the upper edge of the rookery. This platform will be designed in such a manner as to allow for extreme portability, rapid deployment, strength and minimum impact upon the surrounding environment, such as the design outlined in **Figure 2**. While general visual coverage though the use of the remote video system is quite good, the need has been identified to stage a physical presence in this area. Typically, this presence would involve ecological/behavioral studies of Steller sea lions through:

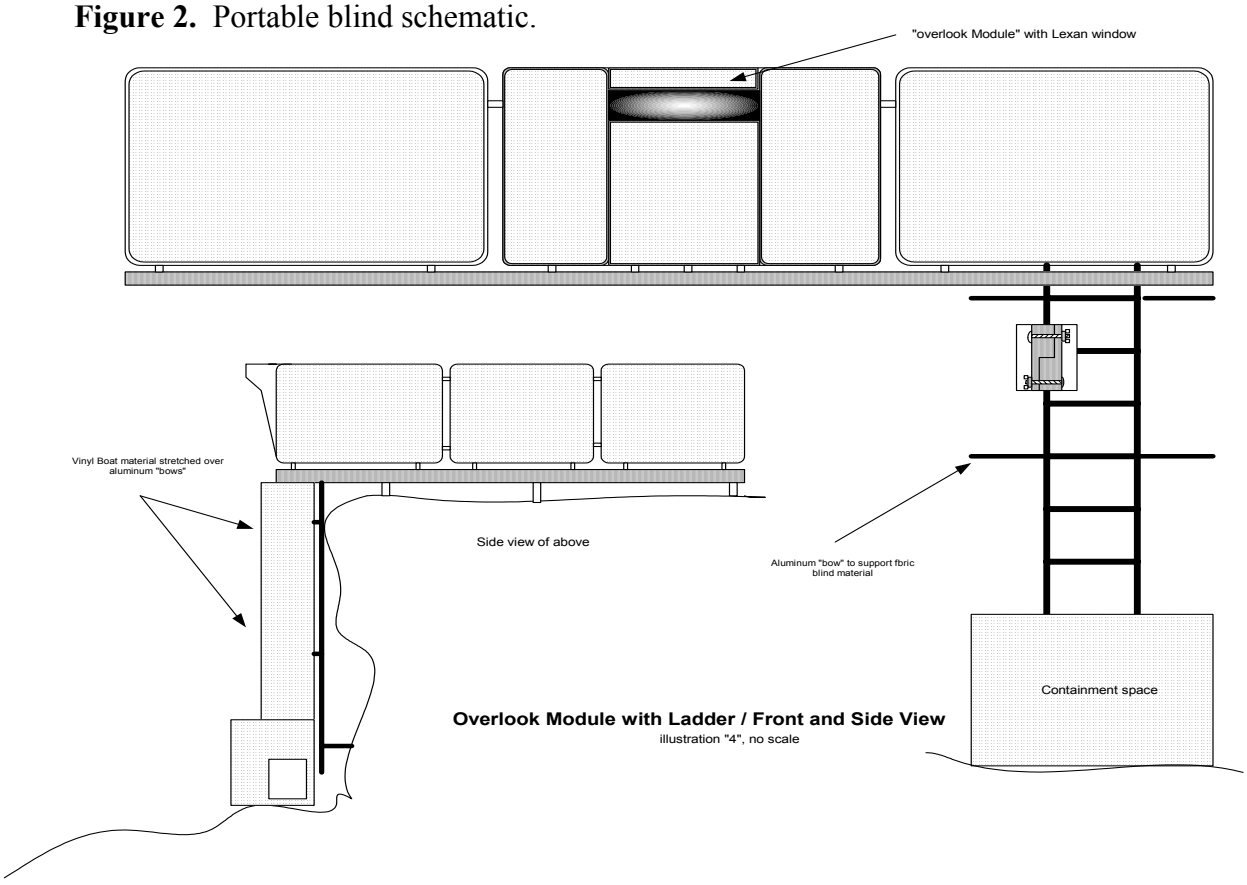
1. direct behavior observation of mother/pup interactions (maternal attendance/investment project)
2. pup branding/post branding work
3. developing techniques for sea lion pup marking
4. facilitating the high-quality photo ID data base of sea lions using the Chiswell Island rookery
5. physiological monitoring of SSL pups during the entire breeding season.
6. observations of transient killer whale presence and predation to the SSL near the Chiswell Island rookery.

Figure 1. Incidental disturbance takes of Steller sea lions at Chiswell Island during 2001 video system maintenance.

Date of Trip	Chiswell Island	
	Present	Disturbed
1/10/01*	20	20
2/1/01*	0	0
2/7/01*	0	0
2/8/01*	0	0
2/22/01*	3	3
3/7/01*	0	0
4/5/01*	0	0
4/16/01+	11	0
5/9/01+	35	12
5/10/01+	15	0
7/31/01+	143	78
9/7/01+	88	40
11/7/01*	46	40
11/24/01*	34	30
Totals	395	223

* Helicopter trip
+ Boat trip

Figure 2. Portable blind schematic.



In addition to the Chiswell video project we also have a funded project to deploy remote video monitoring equipment that transmits video images via satellite to the internet. This eliminates the need for line of sight microwave transmissions and when fully operational will allow deployment of this system at any Steller sea lion haul-out or rookery. We intend to use this system at multiple locations in the Gulf of Alaska. While individual sites cannot be presently identified, potential sites include Cape St. Elias, Sea Lion Rocks and Fish Island. This project will address several objectives of the Steller sea lion Recovery Plan (1992), specifically: conduct visual marking/tagging studies (sections 21, 211 & 443), monitor rookeries for the occurrence of marked animals (section 212), conduct studies on rookeries (section 44), determine age and sex classes of animals on shore (section 441), determine rates of pup production and mortality (sections 442, 512 & 517) and monitor status of tagged animals (section 444).

This Task is funded through the Alaska SeaLife Center / NMFS.

Task 2: Free-ranging juvenile health assessment

One of the leading hypotheses for the cause of the Steller sea lion decline is a reduction in juvenile survival of 10-20% (York 1994). While Steller sea lion pups have been investigated for a number of health indices, including mass (Merrick et al. 1993), body condition (Castellini et al. 1993), morphometrics, skin-fold thickness (Jonker and Trites 2000), hematology (Rea et al. 1998), clinical chemistry (Castellini et al. 1993), immuno-competence (Zenteno-Savin et al. 1997) and pollutant levels (Lee et al. 1996; Saeki et al. 2001), there is no significant difference between 'stable' and 'declining' populations to account for the magnitude of the differences in population trends. Given the lack of any clear physiological difference or indicator of a potential health problem in pups, the concurrent examination of these factors in the age class thought to be most at risk is a logical step.

Through the cooperation of multiple investigators (D. Calkins, J. Mellish, S. Atkinson, P. Tuomi, K. Mashburn, M. Horning, L. Rea, L. Mazzaro), we will thoroughly examine a number of physiological indices in free-ranging Steller sea lion juveniles (1-4 years), including hematology, clinical chemistry, viral serology, immuno-competence, pollutant levels, body condition, genetics and stress indicators.

Basic hematology, clinical chemistry and stress response panels from free-range Steller sea lions will allow us to identify possible infections and/or abnormalities that can affect these parameters. Blood will also be utilized to identify the presence of infection that may cause reduced reproductive performance (e.g., abortion) in later years, such as leptospirosis, chlamydia and herpes. Antibodies to all three infectious agents have been found previously in the blood of Steller sea lions (e.g., Vedder et al. 1987), but these parameters have not been examined extensively or in the past decade of the continuing decline. Large tissue loads of toxic substances such as organochlorines can reduce the effectiveness of the immune system and contribute to reproductive failure in pinnipeds (DeLong et al. 1973, Gilmartin et al. 1976, Reijnders 1987, Hutchinson and Simmonds 1994). While these substances were not found in high levels in preliminary studies of the Steller sea lion, this is an important aspect to monitor in conjunction with other potential health conditions. The measurement of body condition is an essential tool in the overall health assessment of an animal, as it can indicate levels of body energy stores. Animals with low body energy stores are less likely to endure periods of physiological and/or environmental stress. In conjunction with the 3D photogrammetry project (**Task 3.4**), we will monitor the body condition of free-ranging juveniles to assist in the overall assessment of health status. Blood and feces collected from free-ranging juveniles will also be analysed in conjunction

with the transient juvenile program (**Task 3**) for evidence of stress indicators, such as cortisol or corticosterone. High levels of cortisol may indicate a physiologically stressed animal that is unable to utilize body resources in a normal fashion and may be subject to tissue wasting and eventually death. While it has been determined that two genetically distinct stocks of Steller sea lions exist in Alaska (Bickham et al. 1996, 1998) skin samples will be analysed in conjunction with NMML to expand their existing genetic databases. This information will allow us to further determine the amount of genetic diversity between and within stocks.

In association with the life history transmitter project (**Task 3.1**, described below), we will introduce a new experimental paradigm to the assessment of simultaneous effects of all of the above parameters on Steller sea lion population trends. That is, we will be directly testing survivors versus non-survivors within the Western stock, rather than the more common approach of comparing Western stock versus the Eastern stock. The effects of each parameter will be analysed separately as well as combined in a multivariate approach, on individual survival of the very animals tested (survival data coming from life history transmitter units). For life-history transmitter-implanted animals, these parameters can only be assessed at the time of implantation. Data from non-life history transmitter implanted free-ranging animals will provide reference data and data for older age groups, and longitudinal trends in individuals. In addition, data collected under this task will also provide baseline data for body condition trend monitoring via remote imaging.

This study will address multiple objectives of the Steller sea lion Recovery Plan (1992), specifically through the further determination if biological parameters indicate different stocks of sea lions (section 22), monitoring of health, condition and vital parameters (sections 4, 43, 432, 445, 46 & 47), and investigation of sea lion feeding ecology (section 61, 613, 6131 & 6132). In addition, this project will address the recommendations of the Steller sea lion Research Peer Review (1999), through the measurement of body condition and development of new methods for this assessment. Body condition was considered by the Panel to be one of the most important indices to be monitored. This project will also address other suggested areas of research, including the coordination of multiple projects, investigation of parameters within the affected stock rather than the comparison of stocks, manipulative experimental designs and the assessment of juvenile survival rates.

This task is funded through the Alaska SeaLife Center / NMFS.

Task 3: Transient juvenile program

The transient juvenile program will provide a critical resource for numerous projects designed to assess the health status, body condition, stress response, and foraging behavior of juvenile Steller sea lions. Free-ranging juveniles will be captured in water or on land and transported to the Alaska SeaLife Center (ASLC) for short-term research. After a brief period of time (up to 3 months) the animals will be released in the original capture location.

While at the ASLC, these animals will be involved in a number of funded projects, the objectives and details of which are outlined below. The use of wild animals (i.e., not long-term captive individuals) as well as the need for a short-term holding period to complete these studies (i.e., not suitable for field or support vessel holding) makes the juvenile transient program a critical component of these and other studies. Study of the transient juveniles will continue post-release through the use of external tracking devices. This study will address multiple objectives of the Steller sea lion Recovery Plan (1992), specifically through the monitoring of health, condition and vital parameters (sections 4, 43, 432, 445, 46 & 47), and investigation of sea lion

feeding ecology (section 61, 613, 6131 & 6132). In addition, the Steller sea lion Research Peer Review (1999) included recommendations for the increased use of captive animals for research activities, combined with better coordination of multiple programs. The transient juvenile program currently integrates seven peer-reviewed and funded projects with seven principal investigators and six institutions. Rather than each project operating separately, we will greatly minimize the impact on the population though the cooperation of researchers and extensive sharing of samples.

This task is funded through the Alaska SeaLife Center / NMFS and the Pollock Conservation Cooperative Research Center (S. Atkinson and M. Castellini).

3.1. Life history transmitter (LHX) project

A key factor absent from current research and recovery efforts are direct measurements of juvenile Steller sea lion mortality rates. We will specifically measure this parameter through the use of implantable, satellite-linked life history transmitters (LHXs). Mortality transmitters have been used successfully to determine survival rates in a number of free-ranging species, and implants have been shown to be successful in other marine mammals, such as the sea otter (Monnett and Rotterman 2000)

Rawson and Hartline (1964) were the first to use intraperitoneally implanted radio transmitters for the analysis of movement patterns in deer mice, in a pioneering study. Since the study of Neely and Campbell (1973), investigators have mounted efforts to assess the effects of implantation procedures and devices on the behavior and survival of implanted animals. Survival rates have been compared between externally tagged animals, and those equipped with either subcutaneous or intraperitoneal telemetry implants. The papers of Folk et al. (1971), Neely and Campbell (1973), Smith and Whitney (1977), and MacDonald and Amlaner (1980) deliver an excellent overview of the early days of the use of implantable telemetry devices in mammals. The predominant problems in early applications relate to issues of relative size, packaging and sterility of instruments and procedures. Subsequently, recommendations were made for implanted telemetry devices not to exceed 3-5% of animal body mass (MacDonald and Amlaner 1980), even though some authors later found no indications of reduced mobility with implants as large as 10% of animal body mass (Koehler et al. 1987). Modern implantable telemetry tags typically remain under 1% of body mass, a relative size considered to be unproblematic. Packaging and specifically the outermost encasing material have an effect on the likelihood of adhesion of intraperitoneal devices to intestines. This adhesion has been reported as responsible for some of the very few observed complications in intraperitoneal implants (Guynn et al. 1987). Modern inert physiologically compatible resins have resolved this issue (Monnett & Rotterman 2000, C. Monnett, pers comm.). Finally, in the early days some implantation procedures were carried out under “clean but not sterile” conditions (Eagle et al. 1984). Using appropriate instrument sterilization and sterile surgery techniques, infections from implant procedures have become virtually absent. This has reduced the incidence of post-surgical infections to 1 in 160 procedures in sea otters (C. Monnett pers comm.). In 183 yellow-bellied marmots with intraperitoneal implants, 30-day survival and growth rates, as well as pregnancy rates and mean litter sizes did not differ from controls (Van Vuren 1989). In those instances where infections occurred in earlier applications (likely as a result of compromised sterility), bacteremia resulted in deaths within the first week after surgery, in over 90% of cases (Williams and Siniff 1983, Johnson and Berkley 1999, C. Monnett pers comm.). All studies comparing subcutaneous to intraperitoneal implantation concluded that the latter was the preferred technique, generating far

fewer complications than a subcutaneous application (Neely and Campbell 1973, Philo and Follman 1981, Garshelis and Siniff 1983, Agren et al. 2000). As a result, the intraperitoneal implantation of telemetry devices into mammals in general (Marmots: Van Vuren 1989, Silver fox: Bakken et al. 1999, Badgers: Agren et al. 2000), and aquatic mammals in particular (Beavers: Wheatley 1997, River otters: Johnson and Berkley 1999, Sea otters: Monnett and Rotterman 2000), is considered a reasonably routine procedure. Amongst aquatic mammals, 5 deaths attributable to implanted devices have occurred in 366 reported procedures since 1983.

LHX transmitters expand on this approach by providing survival and longitudinal cumulative dive effort data from individual animals, for periods up to 10 years. The LHX will be capable of continuously monitoring five built-in sensors, including pressure, motion, light levels, temperature and conductivity. The transmitter will be able to establish the death of animal, and accordingly time and date stamp the event. When the instrument is exposed to ambient conditions outside of the carcass, all information stored will be transmitted to the ARGOS system via a NOAA satellite. Currently the battery life of the LHX is expected to be a minimum of 8 years, during which time we expect a return of 60-70% of animals tagged, as described below. LHX tags are currently the sole means by which such kind of longitudinal data from individual animals can be obtained covering periods up to 10 years.

This project will address the following major hypothesis:

Ho 1: Juvenile survival does not differ from predicted value for constant population levels.
Ha: (juvenile survival differs from predicted value) supports the conclusion that juvenile mortality (if lower) contributes to the population decline of the Western Steller sea lion stock.

A power test for a one-sided analysis of variance reveals the following probabilities of committing a type II error b (not rejecting Ho when it should be rejected), for a sample size of 72 SMX equipped animals, a=0.05, Ho: annual juvenile survival=0.78 (life table estimate); as well as minimum detectable decrease in survival for b = 0.1

Ha: annual survival: 0.624 0.65 0.702 minimum detection
annual reduction in survival: -20% -16.7% -10% decrease
after one year: b=0.2 b=0.4 b>0.8 -28%
after two years: b=0.01 b=0.02 b=0.026 -4.2%

This assumes amending detections by a correction factor determined to an accuracy of better than 1% from ratio of dual versus single hits from redundant implants. Thus, after monitoring 72 animals for ³2 years, we can detect a decrease in annual survival of as little as 4.2% with a likelihood of b<0.1 of committing a type II error. Greater reductions in annual survival will deliver significant effects at an earlier stage. Monitoring for >2 years will further increase sensitivity.

Figure 3. Implanted telemetry papers by category.

General Technology	Mammals	Aquatic Mammals	Species
Folk et al. 1971, Neely & Campbell 1973, Smith & Whitney 1977, MacDonald & Amlaner 1980			
	Koehler et al. 1987, Rawson & Hartline 1964.		Ord's Kangaroo rat, Montane vole, Deer mouse, Townsend's ground squirrel
	Van Vuren 1989		Yellow-bellied marmot
	Agren et al. 2000.		European badger
	Madison 1980		Meadow vole
	Eagle et al. 1984.		American mink, Franklin's ground squirrel
	Smith 1980 a,b.		White-footed mice
	Philo & Follman 1981		Grizzly bear
	Moe et al. 1995, Bakken et al. 1999		Silver fox
	Guynn et al. 1987, Wheatley 1997, Davis et al. 1984		Beaver
		Ralls & Siniff 1990, Ralls et al 1989, Siniff 1985, Siniff & Ralls 1991, Monnett & Rotterman 2000, Garshelis & Siniff 1983, Williams & Siniff 1983	Sea otter
		Reid et al. 1986, Melquist et al. 1981, Melquist & Hornocker 1979, Hoover 1984, Johnson & Berkley 1999	North American river otter
		Mulcahy & Garner 1999	Polar bear

The new paradigm utilized by the life history transmitter project will allow us to directly examine the characteristics of survivors versus non-survivors. We will specifically monitor two major areas potentially responsible for a reduced juvenile population: 1) dive effort and dive behavior; and 2) body condition and health characteristics. Alterations in diving effort and activity have been linked to changes in seasonal and short-term variation in prey abundance. Dive effort data will allow for a comparison of temporal patterns of foraging effort on a weekly, seasonal and annual basis. In addition, seasonal patterns of mortality may elucidate any potential competition with major fisheries events. In the life history transmitter study, health and body condition assessments will serve two purposes: 1) to determine the immediate and post-operative health status of the individuals; and 2) to directly test the relationship between health status and

body condition with survival versus non-survival outcomes through a multivariate approach. This task will include transient juveniles (selected from Task 3, described above) and free-ranging juveniles (selected from Task 2, described above). A contract team of veterinarians will be assisting in the development and surgical/ procedural aspects of this project. This team will consist of Pam Tuomi (Co-Investigator), Bruce Heath (Veterinary Anesthesia, Ft. Collins, CO), Frances Gulland (The Marine Mammal Center), Marty Haulena (The Marine Mammal Center), Wendell Nelson (Colorado State University), and Terry Spraker (Colorado State University).

This project will address the following objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (sections 4, 43, 432, 445, 46, & 47), assess causes of mortality (section 5), investigate sea lion feeding ecology (section 6, 61, 611 613, 6131 & 6132). In addition, this project provides the new technology required for the determination of juvenile survival rates, as suggested by the Steller sea lion Research Peer Review (1999), and will allow for the comparison within the affected stock, rather than comparisons between stocks.

This sub-task is funded through the NMFS Steller Sea Lion Research Initiative (#NA17FX1429, M. Horning and J. Mellish), the Pollock Conservation Cooperative Research Center (M. Horning and D. Calkins) and the North Pacific Marine Research Council (M. Horning).

3.2. Monitoring of transient juvenile Steller sea lions with external tracking devices.

Researchers first began using externally-attached satellite-linked tracking devices on marine mammals in the early 1980s. Some of the first species attempted were Hawaiian spotted dolphins (*Stenella attenuate*, Jennings and Gandy 1980), bottlenose dolphins (*Tursiops truncates*, Soma and Tsutsami 1986), walrus *(Odobenus rosmarus*, Hills 1987), West Indian manatees (*Trichechus manatus*, Mate et al. 1988), gray seals (*Halichoerus grypus*, McConnell 1986) and crabeater seals (*Lobodon carcinophagus*, Hill et al. 1987). Following improvements in technology in the 1990s, researchers began attaching these instruments to a number of other marine mammal species, such as Northern elephant seals (*Mirounga angustirostris*, DeLong and Stewart 1991), to study dive and foraging behavior, resource utilization, and movement patterns.

Location-only tags (PTT tags) and satellite-linked time-depth recorders (SLTDRs) were first used on pinnipeds in 1990 (Merrick et al. 1994). The first unit to be deployed on a free-ranging Steller sea lion was a 1.0 watt Telonics (Mesa, AZ) ST-4 PTT attached with epoxy glue and mesh to the animal's back. After this unit proved successful, a Telonics 1.0 watt ST-5 PTT was coupled with a Wildlife Computers (Redmond, WA) Type-2 time depth recorder (TDR). This package, in addition to location data, recorded and transmitted dive depths and durations, surface times, and water temperatures. The entire unit measured 100 cm by 17 cm by 3 cm and weighed 800 g in air. Forty-five of these units were deployed in the Gulf of Alaska, Bering Sea, Aleutian Islands, and Kuril Islands between 1990 and 1992.

Since 1990, hundreds of SLTDRs and PTT tags have been successfully deployed on free-ranging Steller sea lions throughout their range, recording hundreds of thousands of dives (e.g., Rehberg et al. 2001, Loughlin et al.1998, Merrick and Loughlin 1997, Calkins 1996, Merrick et al. 1994, Loughlin et al. 1993). Mean deployment periods ranged from 8 to 85 days. Modern SLTDRs from Wildlife Computers weigh between 170 g and 425 g, depending on the battery configuration. These devices transmit data using the Service-Argos system onboard NOAA Tiros-series satellites and can provide geolocation information accurate to within 150 m. The method most commonly used to attach SLTDRs is to glue them mid-dorsally either directly the

hair (Loughlin et al. 1993), or to a mesh patch fixed to the hair using fast-setting epoxy (Merrick et al. 1994). PTT tags, such as Wildlife Computer's Smart Position and Temperature Transmitting (SPOT2) tags, weigh as little as 82.5 g. Because of their size, these units are commonly attached to the top of the animal's head via epoxy and mesh. Since the transmission time on these instruments is much shorter than that of an SLTDR, the head-mounted units allow for more at-sea positions to be transmitted. Both types of units fall off during the annual molt and are generally not recovered.

A critical component of the captive, transient juvenile program and the life history transmitter project is the post-release monitoring of individuals. In tracking released animals, we will be able to assess the success of the captive and implant programs. In addition, we will be able to compare data from short-term captive transient juveniles implanted with life history transmitters with free-ranging juvenile life history transmitter implants. One of the two types of tracking devices described above (SLTDR or SPOT) will be attached to provide dive behavior and location data, depending on the tag utilized.

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): develop methods for non-lethal sampling (section 43), investigate sea lion feeding ecology (section 61, 613, 6131 & 6132).

This sub-task is funded through the ASLC / NMFS.

3.3 Monitoring of stress responses in short-term captive Steller sea lion juveniles.

Quantifying stress in marine mammals has proven to be a significant challenge because of the difficulty in obtaining baseline data from an unstressed state (St. Aubin and Dierauf 2001). Studies have shown that numerous hormones are released in response to a perceived stressor (natural or in captive environment), such cortisol, corticosterone, glucocorticoids and other hormones. Under optimal environmental conditions, stressors are occasional events and corticoid actions on the body are acute and short-lived. When a coping mechanism can be established, the stressful stimuli will be controlled and the effect is not harmful (Goldstein 1995). Sub-optimal or severe conditions can cause chronic release of corticoids and a subsequent chronic stimulation of corticoid-sensitive organs resulting in adverse physiological symptoms.

Because of the importance to commercial meat and dairy concerns, the effects of stress have been most extensively studied as it relates to reproduction in commercially viable domestic animals. Glucocorticoids have been found to interfere with both male and female reproduction in several species of domestic mammals (Lesage et al. 2001, Liptrap 1993). Fewer studies have been done on the effects of chronic stress on the immune system and metabolic function. Quite often effects are occurring at the cellular level and testing systems are just now becoming sensitive enough to detect and elucidate what is actually occurring (Norman and Litwack 1987, Dandona et al. 2001). Chronically elevated corticoid levels suppress the production of immunoglobins by B cells in rats (Munck and Crabtree 1981) and is one of the symptoms of Cushing's disease, which leads to metabolic compromise, including loss of bone density, muscle wasting, growth impairment and diabetes (Ezrin et al. 1973.)

Thyroid hormones act on kidney, liver, cardiac and skeletal muscle tissues that together function to control basal metabolic rate. The two most biologically active circulating thyroid hormones, triiodothyronine (T_3) and tetraiodothyronine (T_4) are secreted in response to thyroid stimulating hormone (TSH) released by the pituitary. TSH is in turn, regulated by the hypothlamic release of thyrotropin releasing hormone (TRH). As T_3/T_4 blood levels fall, TRH stimulates TSH release. With increased metabolic demand, T_3/T_4 release is increased. Blood

samples collected from Steller sea lions during controlled fasting experiments will allow us to investigate how serum thyroid hormone concentrations change in response to food deprivation in an animal which is known to undergo periods of natural fasting in the wild. Female Steller sea lions fast for 1 to 2 weeks during the summer breeding season in order to give birth and nurse their young. Males are also thought to fast while defending their territory during the breeding season. By simulating these fasting bouts in a captive environment the effect of food limitation and complete fasting and the resulting changes in body condition (i.e., total fat content on circulating hormone levels) can be addressed.

It can be assumed that the capture, transport, sampling and short-term captivity at the ASLC will constitute an acute stressor for juvenile Steller sea lions. In order to objectively assess the captive transient juvenile program, these stress hormones will be monitored in an effort to evaluate their physiological response. In addition, we will assess the physiological response to the life history transmitter implantation procedure.

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (sections 432 & 445).

This task is funded through the Alaska SeaLife Center / NMFS, the Pollock Conservation Cooperative Research Center (S. Atkinson) and the NMFS Steller Sea Lion Research Initiative (#NA17FX1429, M. Horning and J. Mellish).

3.4. Assessment of 3D photogrammetry as a tool for estimation of body mass and condition

Body condition is considered one of the most essential indices of an animal's nutritional and health status. However, limited data on the body condition of free-ranging Steller sea lions exists, largely due to the logistical difficulties associated with capture and handling of a large species that inhabits primarily remote locations. The Satellite Linked Data Acquisition and Photogrammetry (SLiDAP) system is currently being developed by M. Horning (Texas A&M University) in cooperation with NMFS and the ASLC. This project includes the installation of multiple remote imaging stations in the Aleutian Island chain (locations as yet to be determined; installation, operation and servicing procedures under NMFS permit #782-1532 to T. Loughlin), to allow for year round age-specific counts at haul-outs and rookeries.

As part of this project, the use of 3D photogrammetry to accurately determine body mass and body condition will be tested and validated. As noted by the Steller sea lion Physiology Workshop Review (1999), an accurate assessment of body condition is of high importance for this species. Preliminary studies conducted by J. Waite and M. Horning suggest that the length and girth of Steller sea lions can be measured to better than 2% accuracy with the use of 3D photogrammetry (Waite & Horning 2000, Waite 2000). Further studies by Castellini (2001) suggest that total body water may be highly predictable through the use of a girth index, which leads to the possibility that morphometric measurements made through 3D photogrammetry will allow for the remote estimation of body mass and body condition. As a part of this study, selected short-term captive transient juvenile subjects (from **Task 3**, described above) will be fasted for brief periods (up to 2 weeks) to determine the capability of the photogrammetric procedure to identify animals of the same age class under a range of body conditions. In addition, this study will examine potential regional patterns of blubber mobilization during periods of fasting which will assist in the development of accurate morphometric assessments. Fasting periods of up to 2 weeks will simulate periods of reduced foraging success in the wild. This task will include free-ranging and transient captive juveniles.

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (sections 4, 43, 432, 445, 46 & 47). In addition, body condition was considered by the Steller sea lion Research Peer Review (1999) to be one of the most important indices to be monitored. This project will directly address the recommendation to develop a reliable, inexpensive index of body condition. It will also directly incorporate the imperative to investigate the physiological response of individuals to starvation, using captive animals. Short periods of food restriction will simulate periods of reduced prey availability and provide essential information as to the coping mechanisms of the Steller sea lion.

This task is funded through the NMFS Steller Sea Lion Research Initiative (#NA17FX1430, M. Horning and J. Mellish).

3.5. Fatty acid signatures of juvenile Steller sea lions

Several projects are already underway targeting the identification of fatty acid composition of Steller sea lion tissues (i.e. blubber) and that of their possible prey species, including field collections by ADF&G, NMML, UAF/GAP, U of Washington and NMFS/Auk Bay. Each of these groups has specific goals for the collection of their subset of the samples, however, the ultimate goal of these investigations is the identification of those prey species that have maximum likelihood of contributing to the mixture of lipids ingested by sea lions in the wild. To be able to accurately model the contributions of these species to the diet mixture, the biochemistry of how sea lions utilize lipids from their diet and how they are incorporated into the energy reserves such as the blubber layer must be evaluated. Controlled feeding studies on phocid seals have shown that the rates of incorporation of fatty acids into the lipid stores can vary significantly, both among specific fatty acids and between species. This suggests that to be successful in developing an accurate model of how prey species contribute to diet, we must undertake captive feeding experiments to determine how Steller sea lions incorporate new dietary fatty acids into the blubber layer and to what degree de novo synthesis of fatty acids influences the fatty acid signature.

This Task also proposes to gain a better understanding of how juvenile sea lions tolerate short periods of food deprivation under natural states of body condition through the examination of fatty acid signatures obtained prior to and after a brief fast. How well are these animals buffered by their body reserves against short-term food limitation in their environment (e.g. due to localized depletion of resources) in their natural state? Does metabolic chemistry change at a different rate or to a different degree in wild (possibly nutritionally disadvantaged) animals compared to animals that have been maintained on a manipulated diet in captivity? In the pinnipeds that have been studied to date, metabolic shifts occur at the end of the fast characteristic of depleted lipid energy stores, even when there is no indication that total body lipid reserves are exhausted (Nørdoy et al. 1992). In an earlier review we hypothesized that this may reflect the depletion of a particular kind of lipid (a particular fatty acid or group of fatty acids) important in energy provisioning (Castellini and Rea 1992) and could result from a selective release of specific fatty acid moieties from the blubber stores during fasting. This capacity for selective mobilization has been shown in emperor penguins (Groscolas 1990), and most recently in Weddell seal pups (Rea et al. 1997). For this study it will be important to determine if there is selective mobilization of particular fatty acids during fasting resulting in a change in the fatty acid composition of lipids. If we can relate changes in the availability of particular important fatty acids to changes in fasting biochemistry seen during each season, we

move towards a better understanding of the factors that are regulating changes in lipid and protein catabolism during the fast. Particularly focus should be given to those periods demonstrating major shifts in fasting biochemistry. One such period of focus would be the shift away from a protein sparing metabolism documented after only 7 days of fasting during previous studies on Steller sea lions. This would significantly improve our understanding of what blood chemistry data collected from free-ranging animals imply about the nutritional status of individuals at the time of capture.

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (section 432 & 445, 46, 47), and investigate sea lion feeding ecology (section 611). It will also investigate the physiological response of individuals to starvation, using captive animals, as specifically suggested by the Steller sea lion Research Peer Review (1999). Combined with the information on body composition collected during the fasting periods (**Task 3.4**), we will obtain essential information regarding the physiological response of juvenile Steller sea lions to reduced prey availability/ fasting.

This project is funded through the Alaska Department of Fish and Game (L. Rea) and through the NMFS Steller Sea Lion Research Initiative (#NA17FX1430, M. Horning and J. Mellish).

3.6. Vitamin requirements of Steller sea lions

Nutritional stress has been identified as having a significant, if not primary, role in the precipitous decline of the Steller sea lion in parts of its range (Marine Mammal Commission Annual Reports). Investigations into this hypothesis have focused on food availability (Merrick *et al*. 1997), caloric composition (Rosen and Trites 2000a), and various energetic concerns arising from both these issues (Rosen and Trites 1999, 2000b). The lower fat content and higher digestive costs of a pollock diet resulted in weight loss in captive animals, lending credence to the argument that dietary shifts necessitated by depleted stocks of energy-rich prey such as herring may be having population-wide effects on Steller sea lions. Though food quality is recognized as an important issue in the consideration of nutritional stress (Rosen and Trites 2000a,c), little attention has been directed to specific nutrients that might bear on health and productivity. Chief among these are the fat-soluble vitamins, which assume particular importance in view of the recognized differences in the fat content between the preferred and present diets of Steller sea lion.

Research into the specific vitamin requirements for marine mammals is sparse (Geraci 1981). In captivity, supplements are generally provided to compensate for a reduced variety of foodstuffs and losses during frozen storage of the diet. Often, the level of supplementation is established empirically, sometimes with negative consequences (Mazzaro et al. 1995a). Of necessity, studies on the metabolic consequences of various diets on Steller sea lions have been conducted on animals receiving multi-vitamin supplements (Rosen and Trites 1999). There is no published information on the vitamin content of the natural diet, the vitamin status of free-ranging Steller sea lions, or the metabolic parameters that define their vitamin requirements. We propose to investigate these aspects of Steller sea lion health with respect to the fat-soluble vitamins A (retinol) and E (tocopherol).

In mammals, vitamin A is essential for reproduction, bone and muscle growth, cell maintenance, and vision. Wald (1968) defined the biochemical role of vitamin A in vision; mechanisms for the role of vitamin A in reproduction, growth, and cell maintenance relate to gene expression following binding to nuclear receptor proteins (Ross 1999). Night blindness

was the first nutritional deficiency disease to be clearly recognized, and as a result vitamin A was the first fat soluble vitamin discovered (NRC 1987). Other consequences of vitamin A deficiency include retarded growth, impaired reproduction (Biswas et al. 2000), and increased susceptibility to infection.

Vitamin E is known principally as an anti-oxidant, but has other metabolic activities as well (Azzi and Stocker 2000). It is essential for normal reproductive function and the prevention of oxidative damage in muscle and fat. Dietary vitamin E influences plasma concentrations which in turn affect concentrations in milk and the amount delivered to the offspring (Baldi et al 2000). In marine mammals, there is no characteristic manifestation of vitamin E deficiency, but Engelhardt and Geraci (1978) noted electrolyte imbalance and abnormal molting pattern in phocid seals maintained on low vitamin E diets for 18 months. Vitamin E deficiency can produce muscle degeneration, steatitis, liver necrosis and anemia (Geraci 1981).

This sub-task addresses the following recommendations/objectives of the Steller sea lion Recovery Plan (1992): monitor health, condition and vital parameters (section 4, 43 & 46). This project is funded through the NMFS Steller Sea Lion Research Initiative (#NA16FX1418, L. Mazzaro).

3.7 Metal toxicity in Steller sea lion cell lines

Steller sea lions are exposed to a variety of contaminants including metals through their environment and diet, and preliminary studies indicate that they bio-accumulate aluminum, copper, mercury, vanadium, and silver (Sydeman and Jarman 1998, Saeki et al. 1999, Saeki et al. 2001). However, the extent of exposure or bioaccumulation has not been assessed, as previous studies are limited either by the number of metals considered (Saeki et al. 1999) or the number of organs considered (Sydeman and Jarman 1998, Saeki et al. 2001). As a result, the understanding metal bioaccumulation in Steller sea lions is very poor. It is possible that some of these metals are at dangerously high levels in other untested organs. For example, in bowhead whales (*Baelaena mysticetus*) cadmium levels in the liver appear to be relatively normal at 11 ppm, but reach kidney levels of 64 ppm, which are still even higher (200-300 ppm) in the cortex of the kidney (Bratton et al. 1997, O'Hara and Woshner 2001). To fully understand the potential threat of metals, it is imperative that we determine levels in the major organs of the particular species.

The relationship between cause and effect with metal bioaccumulation in the Steller sea lion is also unknown. It is not known how much of a particular metal is toxic, nor do we know which metals are most toxic. In humans and rodents, metals have well-established toxic effects on many of the major systems of the body including the respiratory, neurologic, immunologic reproductive and endocrine systems (Amdur et al. 1996, Chang 1996). It is assumed that similar events will occur in sea lions and we extrapolate potential toxic doses. However, the actual effects of metals and the doses that exert these effects on Steller sea lions are unknown and have not been tested, as we lack adequate and appropriate models.

These types of mechanistic investigations require exposing either the sea lions themselves or a cell line derived from them to metals and studying how they exert their effects. However, exposing sea lions is impractical and undesirable, and there are currently no adequate Steller sea lion continuous cell culture models. There are a few cell lines that express Steller sea line immunoglobins, but these are rodent cell hybrids. While useful for producing antibodies, these cells are not useful for mechanistic studies of toxicology or cellular physiology. Therefore, it is necessary to study the toxicity of metals in Steller sea lions to derive continuous cell lines.

Metals are known to be genotoxic in rodents and humans (Amdur et al. 1996, Chang 1996). It may seem reasonable to extrapolate from humans or rodents to marine mammals, but studies have shown clear differences between even closely related species such as mice and rats, and thus any extrapolations to much more divergent species such as Steller sea lions would be tentative at best (Amdur et al. 1996, Chang 1996). Furthermore, it has been shown with marine mammals in particular that there are such significant differences in potency among species and a level that is highly toxic to one species may not be toxic to another. For example, the concentrations of cadmium in the kidney cortex of a bowhead would kill most land dwelling animals, but the bowheads seem unaffected (Amdur et al. 1996, Chang 1996, Bratton et al. 1997, O'Hara and Washner 2001). Clearly, to fully understand how metals and other toxicants affect Steller sea lions and the doses necessary to induce those effects, it is necessary to develop cell lines from them.

This project is funded through the NMFS Steller Sea Lion Research Initiative (co-investigator, S. Atkinson).

Task 4. Opportunistic utilization of Steller sea lion carcass/tissue sources

Carcass analysis can provide a wealth of information for a given species. Age at mortality and potential causes for mortality can be inferred. In addition, carcasses can provide large depot sources of tissues required for laboratory research, reducing the amount of invasive disturbance to the animate population. Steller sea lion carcasses periodically become available through field research teams from NMFS, ADF&G, The North Pacific Universities Marine Mammal Research Consortium, and Alaska Native Corporations.

4.1 Carcass validation of life history transmitter project

The original LHX concept and project plan included two different types of carcass testing approaches: 1) under highly controlled conditions as technical tests and 2) under more realistic conditions intended to simulate deployment scenarios more closely.

Type 1) carcass testing was initiated at The Marine Mammal Center (TMMC) at Sausalito, CA, in 2001 with 3 carcasses of California sea lions, two dry carcasses with dummy transmitters and one wet carcass with the first working prototype. These tests were conducted under exclusion of predators and scavengers (birds, small vertebrates, and most invertebrates). The wet test was conducted in a small pool of stagnant freshwater due to logistical difficulties of working with running saltwater. The combination of these factors resulted in what is likely a very atypical decomposition progress. We therefore aborted these tests because they will not be helpful in determining the likely decomposition process for the intended LHX deployments. The very first functional LHX prototype that was used for this test was operating under different programming than the actual LHX programming: in an effort to combine the test with other types of implant tag testing the transmitter was set to transmit continuously from time of implantation. As a result of this programming it exhausted its batteries before extrusion and termination of these tests.

We have since decided to pursue only the second type of carcass testing. For these more realistic simulations of ultimate LHX tag deployment conditions carcasses with two implanted tags each will be released on beaches (first round) and in open ocean waters (second round). Each of these releases will correspond to a known, simulated mortality event. The scientific purpose of this type of carcass testing is solely to contribute to the assessment of transmitter reliability rate by comparing the ratio of single to dual hits from the two impants, not to validate the basic concept of using LHX transmitters on marine homeotherms. These carcass tests will be

performed on carcasses of up to 15 California sea lions off the coats of California, and opportunistically on up to 15 carcasses of Steller sea lions in Alaska. The test series will continue throughout the LHX study, in particular since the Steller sea lion work will have to be done opportunistically when carcasses become available.

The reliability rate will ultimately be calculated beginning when we will have a sufficient number of returns, and will be updated throughout the project, as the number of returns increases. The required sample size for a determination of the reliability rate to an accuracy of 1% was determined based on power analysis to be a minimum of 40 paired deployment, mortality events.

The following potential effects may result from these actions:
1) Predation of live LHX implanted animals:
2) Scavenging of deceased LHX implanted experimental animals
3) Scavenging of LHX implanted carcasses

For the above listed actions, these scenarios can be envisioned:
1) the carcass or live animal is torn into pieces by the predator / scavenger, and the tags that are free-floating inside the peritoneal cavity are ejected from the animal before they can be consumed. We believe this is by far the most likely scenario.
2) the tags are swallowed as a whole animal / carcass is consumed or are accidentally swallowed as an animal / carcass is partially consumed. We consider this a far less likely scenario. The tags are free floating and opportunistic observation of predation or scavenging events indicate that animals or carcasses the size of sea lions are not consumed whole by potential predators or scavengers. Since the tags are free-floating inside the peritoneal cavity most likely they will be ejected. VHF-linked transmitters of a construction similar to the LHX tags (electronics encased in physiological resins) have repeatedly passed the gastrointestinal tract of marine mammal and seabird species (M. Horning, unpubl. data), without detrimental effects on the test subjects or tags. It is therefore anticipated that if any tags will get swallowed by predators or scavengers, that these tags ultimately pass the gastrointestinal tract or will be regurgitated without negative effects on the animals. The tags are pressure rated to 200 atmospheres (2800 psi).
3) the tags are bitten down on hard by a toothed predator / scavenger as the live animal or carcass is being disassembled or partially consumed. We consider this a very unlikely scenario. In fact, we consider this to be less likely with implanted tags then with externally attached tags. In the unlikely event that a tag is bitten down on hard by a toothed predator / scavenger, the tag may be destroyed. The electronics do not contain any significant amounts of dangerous substances. The batteries do contain very small amounts of lithium, less than 0.5 grams. As a result of a biting event, the battery may short out or the battery casing maybe become punctured. If the batteries are shorted out, they heat up to approximately 50 degrees Celsius (Wildlife Computers, pers. comm.), an uncomfortable but not dangerous temperature due to the small mass of the batteries. The battery casing will expand but not rupture. The battery casing is extremely strong and capable of withstanding pressures in excess of 10,000 psi. The batteries employed in the LHX transmitters are the sole lithium-based batteries to date certified for unrestricted air transportation based on the extremely rigid construction of the casing. The batteries contain less than 0.5 grams of lithium, which eliminates the potential for explosion if the casing were to rupture and the lithium were to come into contact with water.

This task is funded through the Pollock Conservation Cooperative Research Center (M. Horning and D. Calkins), the North Pacific Marine Research Council (M. Horning), and the Alaska SeaLife Center / NMFS.

4.2. Assessment of reproductive failure in Steller sea lions.

Steller sea lions appear to have very low reproductive success compared to other pinnipeds. It is estimated that the majority of the adult females are successfully impregnated each breeding season, but only approximately two-thirds of these females carry their pups to term. Reproductive failures are characterized by re-absorption of the fetus early in pregnancy and abortions later in the term. One suspected cause of pre-term reproductive failure are bacteria such as *Leptospira* and *Brucella*, which have been closely associated with reproductive failure in pinnipeds (Smith et al. 1974, Rhyan et al. 2001). While they have been found in other pinniped species (Bricker et al. 2000, Nielson et al. 2001, Colagross-Shouten et al 2002, Calle et al. 2002), the prevalence of these and other bacteria in the Steller sea lion have not been thoroughly investigated. Opportunistic collection of aborted fetuses will allow us to document and examine this phenomenon, as both *Leptospira* and *Brucella* are commonly found concentrated in aborted fetal and placental tissues in a variety of mammals (Malone et al. 1997, Donahue and Williams 2000, Gidlewski et al. 2000, Rhyan et al. 2001, Guitian et al. 2001) .

Another suspected cause of reproductive failure in Steller sea lions is the role played by environmental contaminants in both reproductive disruption as well as fetal exposures. It has been shown that xenobiotics such as DDT, dioxin, and polycyclic aromatic hydrocarbons have a deleterious effect on adult reproduction and fetal organ development in a wide variety of species (Wiig et al. 1998, Hoyer 2001, Washington et al. 2001, Hoekstra et al. 2001, Akingbemi and Hardy 2001, Moran et al. 2001, Gotz et al. 2001, Safe et al. 2001). Environmental contaminants, PCBs in particular, have been found to interfere with the immune response in laboratory animals through the hyperadrenalcortical effects, causing chronic corticoid release and subsequent suppression of B cells (Munck and Crabtree 1981, Fuller and Hobson 1986). A feeding study of harbor seals that was conducted over a 2 1/2 year period showed there was a positive correlation between high levels of organochlorines and suppression of natural killer cells and specific T-cell activity (de Swart et al. 1993, 1994,1996). Immunosuppressive effects of organochlorines are also indicated in several different studies of striped dolphins affected by the morbillivirus epizootic of 1990 in the Mediterranean Sea (Aguilar and Borrell 1994, Troisi et al. 2000, 2001, VanLouveren et al. 2000). Moreover, because it has been found that organisms will exhibit species differences (often to the level of organ specific) in enzyme systems to metabolize different OC compounds (Passivirta 1991, Boon et al. 1994, Reijnders 1994, Rice and O'Keefe, 1995), generalizations regarding the effects of organochlorines can not be made between species. In other words, a chemical found to be harmful in one species may be readily metabolized and cleared from the system of another. Opportunistically collected placentas will also be examined for basic physiological structure and function, enzymology, contaminant load, immunoglobins and endocrinology. Aborted fetuses and deceased newborns (0-30 days old) will similarly be examined for enzymology, contaminant load and endocrinology.

This task is funded through the Alaska SeaLife Center / NMFS.

4.3 Metal toxicity in Steller sea lion cell lines

This subtask is in conjunction with **Task 3.7**. Please see the description found in **Task 3.7** for full literature review. There are no adequate cell culture models of Steller sea lions, therefore this

task will involve the establishment of continuous cell lines with hTERT, the catalytic subunit of telomerase and E6/E7 the oncoproteins of human papilloma virus. Cell lines will be produced from lung, kidney, liver, skin and reproductive organs. These organs were selected because they represent some of the major toxicities of metals and allow for studies involving interactions between different cell types. These cell lines will be made available to other researchers holding appropriate permits.

This project is funded through the NMFS Steller Sea Lion Research Initiative (co-investigator, S. Atkinson).

Task 5. Development of a floating platform trap method for the capture of Steller sea lions.

Capture and handling of Steller sea lions for collection of biological samples, marking, and instrumentation is essential for monitoring the status of the population and for identifying causes of the population decline or factors that may prevent or delay recovery. Current standard methods of capture include the use of hoop nets on land for pups and juveniles, and the use of a lasso for aquatic capture of older pups and juveniles. While these methods are effective, they greatly limit the number of animals sampled, particularly for the larger age classes. Therefore, we propose to develop the use of floating platform traps for more efficient captures of Steller sea lions in Alaska.

Platform traps have been used successfully for sea lions in Puget Sound, Washington. From 1989 through 2002, NMML personnel have captured over 1,300 California sea lions and over 30 Steller sea lions In Puget Sound, Washington, with no accidental mortality and no severe injuries (NMML permits 835 & 782-1446; Section 109H Marine Mammal Protection Act). Some individuals have been repeatedly captured as many as 8 times. The average handling time for sea lions that are not instrumented is about 10 minutes.

Platform traps for use in Alaska would be patterned after existing models, which consist of a 12-ft. wide buoy with a 12-ft. by 12-ft. platform for a haul-out surface. There are 6-ft. high steel cage walls around the perimeter of the platform, with a wide trap door on one side. Sea lions haul out and return to the water freely through the trap door. To capture sea lions, the trap door is dropped when sea lions are hauled out inside. Captured sea lions are transferred into a holding cage on 30-foot barge that docks with the capture cage, then moved one at a time from the holding cage into a stainless steel squeeze cage. This system is analogous to handling runs and squeeze cages used for livestock on farms and ranches. The squeeze cage restricts the movement of the sea lions without harming them or exposing handlers to unnecessary risk. While an individual sea lion is in the squeeze cage, we can weigh and measure it, attach satellite-linked depth recording instruments, apply brands or tags, draw blood, and collect other biological samples. When all procedures are completed, the squeeze is opened, the animal is released, and the next animal is allowed in. The exact dimensions of our traps will vary depending on materials that are available and on the conditions at particular deployment sites.

The use of floating platform traps has multiple advantages over traditional methods of capture. This method will allow for a larger sample of the population to be monitored, and average handling time for non-instrumented animals is greatly reduced. This technique is non-invasive to the remainder of the population, and will facilitate capture of larger juvenile, and sub-adult and adult sea lions. In addition, this method precludes the need for immobilizing drugs such as Telazol or ketamine hydrochloride. Although the likelihood of accidental mortality with such drugs is not great, individual reactions are unpredictable and there is some risk. The development of these floating traps will be initiated at several sites throughout the Alaskan range

of the Steller sea lion, including but not limited to: Seward, Chiswell Island, Homer, Kodiak, Prince William Sound and Southeast Alaska.

This task is funded through the Alaska SeaLife Center / NMFS.

C. Methods

1. *Justification*

All projects outlined in this permit application are focused on the collection of information crucial for the ongoing Steller sea lion recovery effort. Therefore, the most accurate data will only be available through directed research on this species. The tasks outlined are in response to recommendations from several panels, including the NMFS Steller sea lion Recovery Plan (1992), the Steller sea lion Research Peer Review (Didier 1997a, b), Steller sea lion implant workshop (Horning et al. 1999), the Steller sea lion Physiology Research Workshop Review (Williams et al. 1999), 'Is It Food II' Workshop (DeMaster et al. 2001), and the Steller sea lion Juvenile Capture Workshop (in prep). Samples collected will be shared amongst collaborating projects and investigators to minimize disturbance and handling whenever feasible.

Task 1. Remote video monitoring

Direct observations on haul outs and rookeries have long been essential for detailed investigations of Steller sea lions. The existing Chiswell Island location has provided year-round information on the behavior and structure of the haul-out population which was previously unavailable. The further development of this technology will allow for in-depth studies of Steller sea lions with only minimal disturbance, as detailed from previous studies on Chiswell Island (see Introduction). Therefore, we request authorization to capture and sample **60** pups per year, with an incidental disturbance (for pup handling and video maintenance) of **150** animals per event, for a total of **2,100** individuals incidentally disturbed of all age classes and sexes.

Task 2. Free-ranging juvenile health assessment

Juvenile Steller sea lions are thought to be the age class which may be experiencing the largest amount of mortality, perhaps due to nutritional stress or some other factor. Therefore, it is imperative that we shift the current research focus from pups to the age class thought to be most at risk. The first step to monitor this age class requires a comprehensive evaluation of their health status. While physiological studies of free-ranging pups were able to obtain a sample size in the hundreds, the much larger size and mobility of juvenile Steller sea lions precludes a comparable database. Therefore, we request the authorization to sample **150** juveniles per year, with and incidental disturbance of **3,750** per year, in an effort to maximize information gained while minimizing disturbance to other individuals.

Task 3. Transient juvenile program

As outlined in **Task 2**, juveniles are thought to be the age class experiencing the largest rate of mortality, and therefore research efforts must be directed towards this age class. In an effort to maximize information gained while minimizing disturbance, we have designed a short-term captive research program. This program will allow for multiple investigators to share sampling regimes, samples, and information. This program will also allow for studies that would be otherwise impossible in the wild, given the very low probability and large disturbance involved with re-captures of individual animals. While no imperical data exist to calculate the program size, we request authorization for the capture and holding of **16** juveniles per year (a subset of the **150** takes listed in Task 2), based on the logistical and financial constraints of short-term captive maintenance.

Task 4. Opportunistic utilization of Steller sea lion carcass/tissue sources
Carcasses can provide an invaluable source of information without causing any disturbance to other individuals. It is in the best interest of the species to maximize the amount of information gathered in a non-invasive manner, and therefore we request authority for the opportunistic collection and sampling of **30** placentas, **30** aborted fetuses, **30** neonatal carcasses and **15** juvenile/subadult/adult carcasses per year. Tissues that are not used within the scope of this permit application will be made available to other researchers through the Alaska Regional Stranding Network.

Task 5. Development of a floating platform trap method for the capture of Steller sea lions
One of the most difficult procedures for larger Steller sea lions is that of capture. The use of a floating platform has proven effective for California sea lions and may prove to be a less stressful method of capture for Steller sea lions. Therefore, we request the authorization to develop this capture method, through the capture of up to **240** pups of both sexes, **80** juveniles of both sexes and **80** adult females per year. Of those animals captured, we request the authorization to sample **60** pups of both sexes, **20** juveniles of both sexes and **20** adult females per year.

2. *Duration of the project and locations of taking*: A five-year permit from the date of issuance is requested for the implementation and continuation of the free-ranging and transient research program. We request authorization for research within the state of Alaska, and we anticipate that this research will be conducted primarily within the range of the Western US stock, including the North Gulf of Alaska and the Aleutian Island chain. We request that this permit be valid at all times of the year, as is appropriate for a given task. While the reproductive/pupping season is the traditional period for studying Steller sea lions due to the aggregations of animals and tolerant weather conditions, one of the largest shortcomings of existing data is a lack of information regarding the physiology and ecology of the Steller sea lion outside of this season. Year-round research will allow us to instead utilize the pupping season as a control (given the increased amount of information available), and to examine the species on a larger, more complete yearly scale. Exact dates and locations of the research proposed are unavailable at this time, as they are largely determined by constraints on support vessel time, personnel availability and weather conditions. As the time of an impending action approaches and dates and locations are more finalized, this information will be provided to the Regional Coordinator. It is likely that research will initially be focused on the Gulf of Alaska region, where pup weights appear to be comparably low. Once research projects have been fully developed, they may expand to the Aleutian Island chain.

3. *Types of taking involved and estimate of numbers of animals that would be taken*:
Descriptions of the types of takes involved in this application are listed below. Specific numbers, age classes and types of takes are thereafter listed in association with each respective Task. This information is also summarized in **Table 1**.

Table 1. Annual Task take table**.** Please note that some tasks are based on shared samples and therefore not require additional takes and are not listed in this table.

Task	Activity/Take	# / age class	# takes/animal
Task 1.	**Incidental disturbance**	**2,100 all ages (both sexes)**	
	Capture and sampling: including anesthesia, body mass/morphometrics/ 3D photogrammetry, blood sampled, tissue collection (blubber & skin), hot branded, flipper tagged, fecal collection.	**60 pups** (both sexes)	**1**
Task 2.	**Incidental disturbance**	**3,750 all ages** (both sexes)	
	Capture and sampling: including anesthesia, body mass/morphometrics/ 3D photogrammetry, blood sampled, tissue collection (blubber & skin), body composition, ultrasound, fecal collection, skin and mucosal swab.	**150 juveniles** (both sexes)	**1**
	Life History Transmitter implantation: includes health assessment and dual life history transmitter implantation surgery	**60 juveniles** (from above 150 take) (both sexes) (total 120 over 5 years)	**1**
Task 3.	**Transport and maintenance at ASLC:** includes transport and up to 3 months maintenance at the ASLC	**16 juveniles** (from above Task 2, 150 juvenile take)	**1**
	Initial health assessment: including anesthesia, body mass/morphometrics/ 3D photogrammetry, blood sampled, tissue collection (blubber & skin), body composition, flipper tag, ultrasound, fecal collection, skin and mucosal swab, x-ray, endoscopy, urinalysis.		**1**
	Weekly health assessment: including anesthesia (as deemed necessary by attending veterinarian), body mass/morphometrics/ 3D photogrammetry, blood sampled, ultrasound, fecal collection.		**10**
	Final health assessment: including anesthesia, body mass/morphometrics/ 3D photogrammetry, blood sampled, tissue collection (blubber & skin), body composition, ultrasound, fecal collection, skin and mucosal swab, x-ray, endoscopy, urinalysis. ***takes listed below are additional takes on a subset of these 16 juveniles**		**1**
3.1	**Life history transmitter implantation**: includes dual life history transmitter implantation surgery	**4** juveniles (both sexes)	**1**
3.2	**External tag attachment**: includes attachment of external data logger (SPOT or SLDTR)	**16** juveniles (both sexes)	**1**

3.3	**Adrenocorticotropic hormone challenge:** includes injection of synthetic ACTH, with blood samples taken every 15 minutes for two hours	**4** juveniles (both sexes) (total 10 over 5 years)	**1**
3.4	**Controlled fasting**: includes a 2 week fasting period, mass / morphometrics / 3D photogrammetry, body composition, blubber biopsy, blood sample pre- and post-fast.	**4** juveniles (both sexes)	**1**
Task 4			
4.1	**Carcass collection:** for technical implantation and validation of the life history transmitters (**Tasks 2, 3.1**)	**15**	
4.2	**Collection of expelled placentas** **Collection of aborted fetuses** **Collection of neonatal carcasses** **Collection of major organs from deceased individuals**	**30** **30** **30** **30**	
Task 5	**Capture:** via platform trap method	**240** pups (both sexes) **80** juveniles (both sexes) **80** adults (females)	**1** **1**
	Sampling: including body mass/morphometrics/ 3D photogrammetry, blood sampled, tissue collection (blubber & skin), flipper tagged, fecal collection, skin and mucosal swabs.	**60** pups (both sexes) **20** juveniles (both sexes) **20** adults (females)	**1**
Task 6.	**Unintentional mortality (field studies)** While no mortalities are intended, we request authority for up to 5 mortalities per year. In the event of a mortality, samples will be collected for Task 4 and procedures will be revisited.	**5**	
	Unintentional mortality (ASLC) While no mortalities are intended, we request authority for up to 3 mortalities per year at the ASLC. The team of Investigators, Co-Investigators and Veterinarians will decide upon the suitability of an animal for participation in the transient juvenile program, based on a comprehensive health assessment. In the event that an animal does not pass the final health assessment, we request the authority to pursue the following options: 1) extended care at the ASLC followed by release; 2) long-term captivity at the ASLC if not releasable; 3) identification of another long-term captive location; or 4) euthanasia. In the event that more than 3 animals are deemed non-releasable within the period of one year, we will review and re-evaluate procedures.	**3**	

Types of takes:
Adrenocorticoid challenge- Animals selected for the ACTH challenge will be injected with a synthetic ACTH (e.g., Cortrosyn), followed by blood sampling every 10 minutes for 2 hours. It is expected that a peak in cortisol levels will occur 30-45 minutes post injection.
Mitigation - This procedure does not have to occur with all animals at the same time and may take place while animals are undergoing anesthesia for routine health checks. This procedure has been performed successfully with other pinnipeds (Gulland et al. 1999). This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Anesthesia - A veterinarian or other qualified personnel will anesthetize animals for up to four hours using gas anesthesia. This is the estimated maximum time needed for intubations for gas anesthesia, weighing, taking morphometric measurements, branding, taking blood and tissue samples, surgery, and attaching satellite telemeters and/or video/data recorders. Every effort must be made to make the procedure as stress free as possible by minimizing noise, interruptions or other disturbances. All personnel involved in the restraint, anaesthetic and research procedures should discuss personnel placement, assignments and duties prior to each event. The veterinarian or veterinary technician in charge of anesthesia will remain stationed at the head of the animal during the entire procedure to monitor respiration, depth of anesthesia and vital signs. The anaesthetist will determine when the necessary anaesthetic plane has been achieved and verbally notify the research team when sampling, surgery or other procedures should begin. Research and support staff will communicate with the anaesthesiologist regarding status of manipulations at all times. Animals that can be positioned in sternal recumbency and restrained by use of the squeeze cage bars or a capture box may be masked with isoflurane gas anesthesia using a modified traffic cone held tightly over the face to create an air seal. Care must be taken to avoid injury around the eyes with the cone. Additional restraint of the head may be achieved by using a head board and by placing bumper pads on either side of the head under the squeeze bars to assist in positioning nose forward for placement of the mask. Animals that cannot be adequately restrained for mask induction may be pre-medicated with intramuscular injections of atropine (0.54 mg/ml) dosed at 0.03 ml/kg and followed at least 10 minutes later by Telazol (tiletamine HCl and zolazepam HCl, Ft. Dodge Lab) at a dose of 0.5-2 mg/kg. Midazolam may be used as an alternate drug for sedation at a dose of 0.2 mg/kg IM. It may be possible to reverse some effects of midazolam with an injection of flumazenil (1 ml /10-15 mg midazolam IV or IM). The sedation /preanaesthetic drugs may be administered intramuscularly by jab pole syringe using the standard 14 gauge x 1.57 inch needle (Daninject) and the appropriate volume syringe. Injection sites should be clean and dry and, if possible, cleaned immediately before placement of the needle by rinsing or swabbing with 70% isopropyl alcohol or a dilute solution of providone iodine in sterile saline.

After administration of sedation, the anaesthetist and surgical coordinator will remain with the animal to monitor induction and observe respiration and behavior. Other personnel should remain away from the area, and noise or other disturbance minimized until the animal is fully sedated (about 10 to 20 minutes). Isoflurane gas may be administered for induction at 5% in medical oxygen with a flow rate of 5 to 10 liters per minute from a properly cleaned and calibrated vaporizer (Fluotec II) in a closed circuit gas anesthesia machine via the traffic cone mask. Depth of anesthesia will be judged by the anaesthetist based on respiratory rate and volume, response to stimuli, palprebral reflex, capillary refill, and jaw and muscle tone, and maintained using 1% to 3% isoflurane in 5-10 liters of oxygen per minute flow rate as needed.

Intubation with an appropriate sized (10-16 mm) cuffed endotracheal tube should be utilized for continued administration of isoflurane gas and oxygen whenever possible but may be omitted for very short procedures (less than 10 minutes) or when otherwise contraindicated.

Respiratory and heart rate, oxygen saturation and deep rectal body temperature should be monitored during all anaesthetic procedures and recorded on a written anaesthetic record sheet indicating time and duration of anesthesia, rate of isoflurane and oxygen administration, procedures performed, drugs or other products administered, and reactions of the animal from induction through anaesthetic recovery. Hypothermia (deep rectal temperature < 92 ° F) will be prevented by use of the warm water heating of the cage floor, application of warm (100-105 °F) water bags to flippers and body, drying of the fur and covering the animal with thermal insulating blankets. Hyperthermia (deep rectal temperature > 106°F) can be controlled by wetting the flippers with cool water, applying ice or cold water packs.

An emergency kit will be present at all times consisting of a respiratory stimulant (doxepram), a cardiac stimulant (epinephrine), a parasympatholytic agent (atropine) and a corticosteroid (dexamethasone). Positive pressure oxygen ventilation utilizing the endotracheal tube and a 1 to 5 L re-breathing bag or an assisted respiration bellows system on the anaesthetic machine will also be available if needed.

Administration of isoflurane gas will be discontinued as soon as possible after the completion of necessary research procedures. Oxygen will be administered for several additional minutes until the endotracheal tube can be removed (as judged by the return of jaw tone and swallowing reflexes). The animal will be monitored by the anaesthetist for vital signs and body temperature until it regains voluntary mobility and is ready for release.

Mitigation - To avoid respiratory distress, ischemia (restricted blood flow), or nerve damage, animal's will be properly positioned, i.e. ventrally recumbent, during anesthesia. It is important to avoid prolonged breath holding during gas anesthesia as this can result in cardiac hypoxia (lack of oxygen to the heart muscle): therefore, respiration and blood oxygen saturation will be monitored and oxygen administered as needed. Veterinarians will be prepared to control or assist ventilations when using valium/ midazolam, isoflurane, or tiletamine/ ketamine / Telazol. The animal's body temperature will be closely monitored and steps taken to avoid hypo- and hyperthermia (e.g. cooling with water or covering to keep warm, as necessary). This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Blood sampling – Samples will not exceed 1 cc per kg body mass at time of sampling for all animals to be taken only once. For animals scheduled for multiple sampling, blood volume collected at each take will not exceed the lesser of: a) 1 cc per kg body mass, or b) 5% of total blood volume per month (based on animal mass at the time of collection, as per ASLC protocol request, MMPA #881-1443, and per Murray 2000). The most common site for blood collection in SSL is the caudal gluteal vein, which is near the animal's tail, just to the side of the spine. To locate a vein, the animal must be restrained symmetrically, lying on the stomach with foreflippers tucked against the body, hindflippers straight out behind the animal. There is a small risk of infection associated with penetration of the animal's dermis by the needle. Multiple attempts to obtain a blood sample are stressful and cause some degree of pain, damage to the vein, clotting, bruising and abscess.

Mitigation - To reduce the risk of infection, only clean, sterile disposable needles will be used to obtain blood samples and a new needle will be used for each blood collection. Needles will not be re-used on individual animals or between animals. The area to be sampled will be thoroughly

disinfected with ethyl alcohol or betadine prior to insertion of the needle. Sufficient pressure and/or dry gauze will be applied to the venipuncture site after removal of the needle to minimize the potential for hematoma formation in the surrouding tissues. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.
An emergency kit with equipment and supplies for responding to complications or emergencies should be readily available.

Blubber / adipose biopsy - We will collect a blubber biopsy (up to 0.5 g) from all animals captured for physiological and toxicological analysis. The most common site for blubber biopsy in Steller sea lions is at the base of the neck, or dorsal loin region. There is a small risk of infection associated with penetration of the animal's dermis with a scalpel/biopsy punch tool to obtain subcutaneous blubber.
Mitigation - To reduce the risk of infection, only clean, sterile disposable scalpel blades or biopsy punch tool will be used to obtain biopsy samples and a new scalpel blade or biopsy punch tool will be used for each biopsy. Scalpel blades and biopsy punch tools will not be re-used on individual animals or between animals. The area to be sampled will be thoroughly disinfected with ethyl alcohol or betadine prior to insertion of the cutting instrument. After the biopsy is taken, the wound (about 2 cm long) may be closed with surgical glue (e.g., Vetbond) or suture, as determined by the individual performing the procedure. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Body composition (isotopes / BIA) – Body composition will be assessed through the use of istopically labeled water (outlined in Iverson et al. 1993, Mellish et al. 1999a), and bio-electrical impedance (described in Castellini 2001). Both of these methods have been successfully utilized with pinnipeds to provide a non-invasive measure of body composition. Bowen and Iverson (1998) provides a review of the use of isotope dilution in pinnipeds. Discomfort may result from blood samples required (isotope dilution) and placement of BIA transducers. However, this is only temporary.
Mitigation – As described with blood sampling, only sterile needles will be utilized, and each needle will only be used once prior to disposal. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Branding – Hot branding will be performed according to standard procedures outlined in Merrick et al. (1996). Hot brands place permanent, unique numbers and/or letters on the animal's right and left flanks to improve the ability to identify the animal. Compared to flipper tags, hot branding is a permanent marker that is not susceptible to tag loss. Hot branding is conducted on anesthetized animals to minimize pain. There is the potential for infection at the wound site, particularly because the environment on the rookery is not aseptic and because the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound. There is no quantitative information on the rate of infection caused by hot branding Steller sea lions.
Mitigation- Only qualified veterinarians or other personnel with sufficient experience in the technique will be allowed to perform this procedure. The animal will be anesthetized with isoflurane and respiration will be closely monitored (see anesthesia above).

Capture & Restraint- We will capture Steller sea lion pups on land using hoop nets. We will capture juvenile Steller sea lions of both sexes using underwater lasso. The lasso method has been utilized successfully by NNML, ADF&G and in development at the ASLC. The method

consists of two or three divers, supported by a skiff and a larger vessel, approach a haul-out under water. The natural curiosity of young sea lions draws them to the divers. After a brief period of acclimation, sea lions will approach close enough that a rope lasso tended by personnel in the skiff can be placed around them, slightly anterior to the fore flippers, by the divers. The lasso is tightened and the rope is retrieved by the skiff crew. Animals are wrapped in a restraining net and pulled into the skiff and restrained by hand. This technique has proven to be effective and safe for divers and captured animals. The greatest danger to animals is accidental drowning once the lasso is around them. Only NMFS and ADFG personnel with experience using this capture technique will be used. In addition, we will utilize the platform capture method outlined in **Task 5** for the capture and handling of juvenile, sub-adult and adult Steller sea lions.
Mitigation: To minimize the effects of handling SSL, we will use veterinarians and experienced biologists to watch for signs of distress, and release animals showing such signs. In addition, any animal showing signs of distress while being handled will be released immediately and closely monitored. An emergency kit with equipment and supplies for responding to complications or emergencies will be readily available. There is a risk of accidental death during capture and anesthesia. In addition, some animals could die during disturbance of the rookery, capture and handling, and infection from blood and tissue sampling. We will minimize disturbance to the rookery by working around the margins and avoid going into dense aggregations of animals. The risk of infection will be reduced through the disinfection of sampling sites and the use of sterile/aseptic equipment and sampling techniques. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Endoscopy – This procedure will only be performed while the animal is under anesthesia. The animal will be positioned in sternal or lateral recumbency. The endoscope will be passed into the esophagus and stomach using a stomach tube or sheath if needed to overcome resistance from pharyngeal sphincters. Fluid will be aspirated from the stomach, after which the stomach may be inflated slightly with room air to facilitate visual inspection and possibly identify solid materials. Samples will be analysed for parasites, digestive enzymes.
Mitigation – Endoscopy is an invasive procedure and therefore will only be performed while the animal is under anesthesia. The endoscope and all relevant equipment will be thoroughly disinfected and sterilized prior to each use. Only qualified veterinarians or other personnel with sufficient experience in the technique will be allowed to perform this procedure.

External tag attachment – External data loggers/ satellite tags will be attached via epoxy or neoprene rubber cement to the fur of juveniles, along the dorsal midline above the shoulders.
Mitigation - The use of a slow-setting epoxy minimizes the potential of burning the skin. Only small amounts of epoxy and neoprene rubber cement are applied to the fur. Animals do not typically show a reaction to such small devices, and therefore no major adverse reactions are anticipated. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Fecal collection - Naturally excreted fecal material will be collected opportunistically.
Mitigation - There are no anticipated adverse effects on the animal associated with this procedure.

Flipper tag - Any animal captured will be marked with plastic cattle ear tags for future identification. These tags will be affixed through a foreflipper in loose skin anteriorly, near the area where the flipper meets the body. The hole is made with a punch. Each animal receives two tags, one per flipper, to minimize the chance of losing the ability to identify the animal should one tag be lost. These types of tags are best considered semipermanent markers as they can and do pull out because sea lions use their foreflippers in both aquatic and terrestrial locomotion. When the tag is affixed there is the potential for infection at the wound site, particularly because the environment on the rookery is not aseptic and because the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound. There is also the potential for infection when a tag pulls out of the flipper, for whatever reason. In moving about on a rookery or haulout, or swimming, there is the potential for a tag to be torn out of the flipper by abrasion on the substrate or by hydrodynamic pressure. There is no quantitative information on the rate of infection caused by flipper tagging Steller sea lions.
Mitigation - Care will be taken to avoid placing the tag so low as to have the animal walking on it or so high as to have it irritating the animal's flank area. To reduce the risk of infection, the area will be thoroughly disinfected with ethyl alcohol or betadine prior to applying the tag. In addition, the tags will be thoroughly cleaned and disinfected immediately prior to application. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Morphometrics and 3D photogrammetry – Standard morphometrics will be taken, including mass, length and girth. These procedures require the temporary restraint of an animal, but otherwise have no adverse effects on the individual. 3D Photogrammetry will be performed as described in Waite (2000), with simultaneous images collected from digital cameras operated at four points around the animal.
Mitigation – There are no adverse effects anticipated with these procedures. All efforts will be made to avoid the head region of the animal to reduce potential visual stress. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

*Surgical implantation (**Task 3.1** Life history transmitters)* – Life history transmitter implantation will be performed with a minimum of three people: a surgeon, an anaesthetist and a non sterile surgical assistant. Standard aseptic surgical technique will be practiced, including an appropriate cap and mask and a sterile barrier surgical gown and gloves. The following includes a potential protocol for the surgery, however, this method is still in development and may be modified to minimize the effects on the animal. The surgical site will be prepared by clipping hair, skin disinfection and the use of a sterile drape. The transmitters will be gas-sterilized utilizing ethylene oxide gas (EO) in suitable packaging permeable to gas but not to bacteria. Gas sterilized transmitters should be allowed to outgas for a period of 24 hours before implantation. Surgical instruments and moisture barrier surgical drapes will be purchased pre-sterilized or thoroughly washed, dried, packaged and sterilized in an autoclave or using EO gas. The animal will be positioned securely on the surgical table in dorsal recumbency. A warm water flow under the table and thermal insulating pads covering the surgical table will retard heat loss. Anesthesia will be monitored by use of a respiratory or cardiac monitor. The surgical site will be between the caudal sternum manubrium and the pubic bones, palpated through the abdominal wall. An area - 8 to 10 cm long and 4 cm on either side of the midline should be clipped and hair removed. The skin will be repeatedly scrubbed with alcohol (90% isopropyl) alternated with

providone iodine on clean gauze sponges. A nonporous sterile fenestrated drape will be placed over the surgical site and held with towel clamps. The skin will be incised along the ventral midline, the subcutaneous layer and blubber are sharp dissected. The linea alba will be lifted with forceps to permit penetration of the abdominal wall with a scalpel blade. The linea alba is then sharp dissected with blade or scissors, avoiding the viscera, to a length sufficient to pass the transmitter body (approximately 7-8 cm). Alternately, a skin incision will be made parallel to the long axis of the body in the paralumbar fossa (ventral to the lumbosacral muscles and anterior to the origin of the sartorious muscle). The incision should be extended with sharp dissection through the subcutaneous and blubber layers and through the superficial layer of the lumbodorsal fascia. When the muscular abdominal wall (transverse abdominal muscle) is reached, the fascial layer should be incised parallel to the muscle fibers for 1 to 2 cm and blunt dissection used to enlarge the opening through the muscle and peritoneal layers sufficiently to insert the transmitter (diameter 5.5 cm). The abdominal wall will be grasped on either side of the incision with tissue hooks and lifted up and laterally while the transmitter is inserted through the incision into the abdominal cavity or a tapered trochar will be used to dilate the peritoneal opening and introduce the transmitter. Bleeding will be controlled with hemostatic forceps and ligatures of 2-0 absorbable monofilament suture or with electrocautery. The surgical incision will be closed in layers using absorbable suture in a simple interrupted or mattress pattern. The skin will be closed using a subcuticular pattern of absorbable suture and over sewn with a simple interrupted pattern of non-absorbable suture on a reverse cutting needle. The skin incision will be further secured by the application of surgical glue or staples. Oxygen supplementation will continue until the animal recovers sufficiently to allow removal of the endotracheal tube.
Mitigation – Animals will be monitored at all times by a team of qualified veterinarians. As outlined in the procedures (above), all attempts to maintain a clean, aseptic and whenever possible, sterile environment. Only qualified veterinarians or other personnel with sufficient experience in the technique will be allowed to perform this procedure. An emergency kit with equipment and supplies for responding to complications or emergencies will be readily available. Any animal displaying evidence of infection (swelling, wound discharge, changes in appetite) will be treated with antibiotics or additional surgery as needed and recommended by the attending veterinarian.

Skin punch – Skin samples (2 x 50 mg) will be collected from each animal captured for multiple research projects. Skin will be collected using a 6 mm punch tool, either prior to tag insertion (see above) or from the webbing between the hind flipper. There is the potential for infection at the wound site, particularly because the environment on the rookery is not aseptic and because the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound.
Mitigation - To reduce the risk of infection, the area will be thoroughly disinfected with isopropyl alcohol or betadine prior to applying the punch tool and inserting the flipper tag. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Skin / mucosal swabs – Swabs will be performed for multiple physiological studies. There is a very small risk of infection associated with swabbing the animal's dermis, rectum, and ocular area.
Mitigation: To reduce the risk of infection, only clean, sterile disposable swabs will be used.

This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Ultrasound – A portable ultrasound unit will be utilized to record blubber depth from all captured animals. Blubber will be measured from multiple sites, including the neck, shoulder region and hind quarters. This procedure involves the application of water or alcohol to the fur, followed a momentary light pressure on the skin.
Mitigation - No adverse reactions are anticipated to this procedure, however, This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Urinalysis - Urine may be collected through voluntary urination or catheterization. Voluntary urination is highly unlikely in non-conditioned animals, and therefore is not likely to occur in the scope of this permit application. Catheterization will be performed through the use of a sterile, lubricated speculum to find the urethral orifice. After location, an appropriate length of sterile catheter material (14-18 French, rubber or polypropylene) into the bladder.
Mitigation – Collection through voluntary urination would cause no discomfort to the animal. However, catheterization requires that an animal be under anesthesia. Only sterile technique and equipment will be used to minimize the possibility of infection from catheter insertion. Only qualified veterinarians or other personnel with sufficient experience in the technique will be allowed to perform this procedure.

X-ray – A mobile x-ray unit will be used to examine all extremities, the head and neck, chest, abdomen (anterior and mid) and the pelvis. All images will be taken from a dorsoventral and lateral view. This procedure requires that the animal be under anesthesia.
Mitigation – The primary discomfort associated with this procedure is immobilization, which is removed with the use of anesthesia. Only qualified veterinarians or other personnel with sufficient experience in the technique will be allowed to perform this procedure.

Takes by Task

Task 1. Remote video monitoring

This task involves the installation of satellite-linked and microwave linked remote monitoring equipment on haul-outs and rookeries throughout the Gulf of Alaska. In addition, a portable platform blind will be placed at the Chiswell Island rookery. For this task we request the following takes:
a. A total of **2,100** individuals annually of all age classes and both sexes will be taken by disturbance and temporary displacement into the water. The frequency of disturbance will not exceed 14 times per year, with approximately **150** animals disturbed per event.
b. A total of **60** pups (up to 3 months of age; i.e., captured June through August) annually on the Chiswell Island rookery will be subject to:

i. capture via hoop net
ii. gas anesthesia or reversible narcotics
iii. body mass / morphometrics / 3D photography
iv. blood collection (no greater than 1cc per kg body mass)
v. blubber biopsy
vi. fecal collection
vii. branded
viii. flipper tag

ix. skin punch for genetic and cell culture

Body mass / morphometrics / 3D photogrammetry will be utilized to determine general size and condition of the animal. Blood will be collected to examine the following parameters: hematology, clinical chemistry, viral serology, immuno-competence and stress indicators. Tissue will be analysed for pollutant levels (blubber) and genetics (skin). Flipper tags will be inserted for identification purposes. Ultrasound will be utilized to determine blubber depth, as a measure of overall condition. Fecal samples will be analysed for evidence of parasitic infections and for stress indicators.

Task 2. Free-ranging juvenile health assessment

This task is aimed at the accurate collection of serological, immunological and physiological data from juvenile Steller sea lions. Therefore, free-ranging juveniles will be captured in water, on land, or via floating capture trap and sampled for blood, tissue and body condition. Animals will also be flipper tagged for future identification. The following takes will be involved in this process:

a. A total of **150** juvenile Steller sea lions (1-4 years of age) of both sexes will be captured on land or in the water each year. An additional **3,750** individuals of both sexes and all age classes will be taken through incidental disturbance associated with these captures each year. All captured juveniles will have the following takes:

i. gas anesthesia / reversible narcotics
ii. body mass / morphometrics / 3D photography
iii. blood sampling
iv. tissue biopsy (500 mg blubber, 50 mg skin)
v. flipper tag
vi. body composition
vii. ultrasound
viii. fecal collection
ix. skin and mucosal swabs

Body mass / morphometrics / 3D photogrammetry will be utilized to determine general size and condition of the animal. Blood will be collected to examine the following parameters: hematology, clinical chemistry, viral serology, immuno-competence and stress indicators. Tissue will be analysed for pollutant levels (blubber) and genetics (skin). Flipper tags will be inserted for identification purposes. Body composition will be assessed to determine the energetic condition of the animal. Ultrasound will be utilized to determine blubber depth, as an additional measure of overall condition. Fecal samples will be analysed for evidence of parasitic infections and for stress indicators. Skin and musocal swabs will be analysed for epidemiology.

b. Up to **60** individuals will be selected annually for life history transmitter implantation (see **Task 3.1** for method details). A total of **120** individuals will be utilized for this task over the total 5 year period requested. These animals will undergo health assessments (as outlined below in **Task 3**) and dual life history transmitter implantation upon transport to a support vessel (see **Task 3.1** below for details). These individuals will be released into the area of initial capture after completion of health assessments and implantation surgery.

Task 3. Transient juvenile program

This program will allow for multiple investigators to share sampling regimes, samples, and information. This program will also allow for studies that would be otherwise impossible in the

wild, given the very low probability and large disturbance involved with re-captures of individual animals.

a. Up to **16** juveniles (1-4 years of age) (selected from the **150** individuals captured annually in **Task 2**) will be selected annually for short-term captive research at the Alaska SeaLife Center. Juveniles for the transient program will be selected based upon the research to be conducted and therefore cannot fully be described herein. As a rule we will select healthy animals as determined by initial on-board screening. However, for some studies, animals that are not necessarily the healthiest animals available may be used in the transient program. The on-site team will have to make a judgment call as to whether an individual sea lion is likely to be able to survive the research, such as is always done when selecting research animals. Field tests are under way to determine physiologically if an animal is still suckling but have not been developed yet. Currently we can tell if an animal is weaned by looking at size and eruption and wear patterns in the teeth. Larger animals (larger than 100 kg) and animals that have canine teeth longer than teeth on either side are considered older than 1 year and should be weaned. Previously branded animals will be used if captured. No more than **4** individuals will be chosen at one time, to be held be held in quarantine at the ASLC for a maximum of three months. Please see **Section 3.b.(8)** for a description of the quarantine program. All individuals will be acclimated for up to two weeks in the quarantine environment prior to any invasive research procedures.

b. All **16** juveniles chosen annually for the transient captive program will have the following takes (shared samples indicated in parentheses):

i. initial health assessment, including:

1. gas anesthesia / reversible narcotics
2. body mass / morphometrics / 3D photogrammetry (**Task 3.4**)
3. blood sampling (**Tasks 3.3, 3.5, 3.6, 3.7**)
4. tissue biopsy (blubber ≤ 500 mg and skin ≤ 100 mg; **Tasks 3.5, 3.6, 3.7**)
5. flipper tag
6. body composition (**Task 3.4**)
7. ultrasound (**Task 3.4**)
8. fecal collection (**Task 3.3**)
9. skin and mucosal swabs
10. endoscopy
11. urinalysis
12. x-ray

Body mass / morphometrics / 3D photogrammetry will be utilized to determine general size and condition of the animal. Blood will be collected to examine the following parameters: hematology, clinical chemistry, viral serology, immuno-competence and stress indicators. Tissue will be analysed for pollutant levels (blubber) and genetics (skin). Flipper tags will be inserted for identification purposes. Body composition will be assessed to determine the energetic condition of the animal. Ultrasound will be utilized to determine blubber depth, as an additional measure of overall condition. Fecal samples will be analysed for evidence of parasitic infections and for stress indicators. Skin and mucosal swabs will be analysed for epidemiology. Endoscopy will be utilized to determine internal parasites, stomach enzymes, and potentially diet. Urinalysis will be performed to determine uric chemistry, sedimentation for cellular content and bacterial crystals. X-ray will be performed to determine the condition of the animal (e.g., broken bones, abnormalities).

ii. weekly health assessments, including:

1. anesthesia/reversible narcotics (as deemed necessary by the attending veterinarian)
2. body mass / morphometrics / 3D photogrammetry (**Task 3.4**)
3. ultrasound (**Task 3.4**)
4. blood sampling (**Tasks 3.3, 3.5, 3.6**)
5. fecal collection (**Task 3.3**)

Body mass / morphometrics / 3D photogrammetry will be utilized to determine general size and condition of the animal. Blood will be collected to examine the following parameters: hematology, clinical chemistry and stress indicators. Ultrasound will be utilized to determine blubber depth, as an additional measure of overall condition. Fecal samples will be analysed for evidence of parasitic infections and for stress indicators.

iii. final health screening, including:

1. gas anesthesia / reversible narcotics
2. body mass / morphometrics / 3D photogrammetry (**Task 3.4**)
3. blood sampling (**Tasks 3.3, 3.5, 3.6**)
4. tissue biopsy (blubber $\leq$ 500mg and skin $\leq$ 100mg)
5. body composition (**Task 3.4**)
6. ultrasound (**Task 3.4**)
7. fecal collection (**Task 3.3**)
8. skin and mucosal swabs
9. endoscopy
10. urinalysis
11. x-ray

Body mass / morphometrics / 3D photogrammetry will be utilized to determine general size and condition of the animal. Blood will be collected to examine the following parameters: hematology, clinical chemistry, viral serology, immuno-competence and stress indicators. Tissue will be analysed for pollutant levels (blubber) and genetics (skin). Flipper tags will be inserted for identification purposes. Body composition will be assessed to determine the energetic condition of the animal. Ultrasound will be utilized to determine blubber depth, as an additional measure of overall condition. Fecal samples will be analysed for evidence of parasitic infections and for stress indicators. Skin and mucosal swabs will be analysed for epidemiology. Endoscopy will be utilized to determine internal parasites, stomach enzymes, and potentially diet. Urinalysis will be performed to determine chemistry, sedimentation for cellular content and presence of bacteria. X-ray will be performed to determine the condition of the animal (e.g., broken bones, abnormalities).

Multiple blood collections listed in this Task (**Tasks 3, 3.1-3.7**) involving these individuals will not cumulatively exceed 5% of total blood volume per month, based on animal mass at the time of collection (as per ASLC protocol request, MMPA #881-1443, and per Murray 2000). Major invasive procedures (e.g., surgery) will not be performed within a period of one week prior to release, as has been determined sufficient for healing time in other implanted pinnipeds (M. Haulena pers comm., Lander et al. 2001). In fact, it has been shown that pinnipeds undergoing surgical implantation procedures can be released successfully immediately post-implantation (M. Haulena pers comm., Lander et al. 2001). Animals will not be sedated / undergo anaesthesia within 24 hours prior to release. Minor procedures (e.g., blubber biopsy,

blood sampling) may be performed within 1 hour of release. There will be an attending veterinarian for all procedures to be performed during the short-term captivity of the juveniles.

At the implementation of this program, the team of Investigators, Co-Investigators and Veterinarians involved will decide upon a panel of health criteria (detailed in **Section IV.C.3**) for the participation of an animal in the captive, transient program. In the event that an individual becomes ill during the captivity period, the individual will be remanded (at the discretion of the attending veterinarian) to the health care unit at the ASLC to receive medical care and support. The animal will not be considered a research subject at this time and will not participate in any studies. The animal will be returned to the captive research program and/or released at the discretion of the attending veterinarian.

In the event that an animal is deemed non-releasable at the final examination, we request authorization for the following options:

1) Extension of the short-term period of captivity at the ASLC for additional veterinary care
2) Maintenance of the animal in long-term captivity at the ASLC
3) Identification of another location for long-term captivity
4) Euthanasia.

If more than **3** animals are deemed non-releasable within the period of one year, we will review and re-evaluate the process.

The following subtasks involve juveniles selected for the short-term juvenile captivity program. **All animals listed in Tasks 3.1-3.7 are a subset of the 16 animals chosen annually for this program. Additional takes (i.e., other than those outlined above in Task 3) are listed below for each subtask. Please note that Tasks 3.5, 3.6 and 3.7 do not require any additional takes, and therefore only methods of analysis are shown.**

Task 3.1. Life History Transmitter (LHX) project

a. A total of **4** juveniles will be selected annually for life history transmitter implantation. Each animal will be implanted with 2 transmitters to determine the rate of single transmitter success. No more than **3** animals per group will be selected for this category. Surgery for all animals undergoing the LHX implantation procedure will be performed by a trained contract veterinary team, including Drs. Pam Tuomi (ASLC), Bruce Heath (Veterinary Anesthesia, Ft. Collins, CO), Frances Gulland (The Marine Mammal Center), Marty Haulena (The Marine Mammal Center), Wendell Nelson (Colorado State University), and Terry Spraker (Colorado State University). In cooperation with Texas A&M University's Laboratory for Applied Biotelemetry & Biotechnology, Wildlife Computers is currently testing two variants of the LHX device: a standard tag (**Figure 4**), which only records time and date of death but no dive data, and an enhanced tag, which records and later transmits previously stored cumulative dive effort data, as well as date and time of death. Both tag types are built with identical controller boards and ARGOS-compatible 1 Watt transmitters. The standard LHX tags record and later transmit time and date of death, as well as the temperature profile across a mortality event. The enhanced LHX tags additionally record and transmit weekly cumulative dive effort data such as number of dives, median depth of dives and cumulative vertical travel distance, a dive effort indicator.

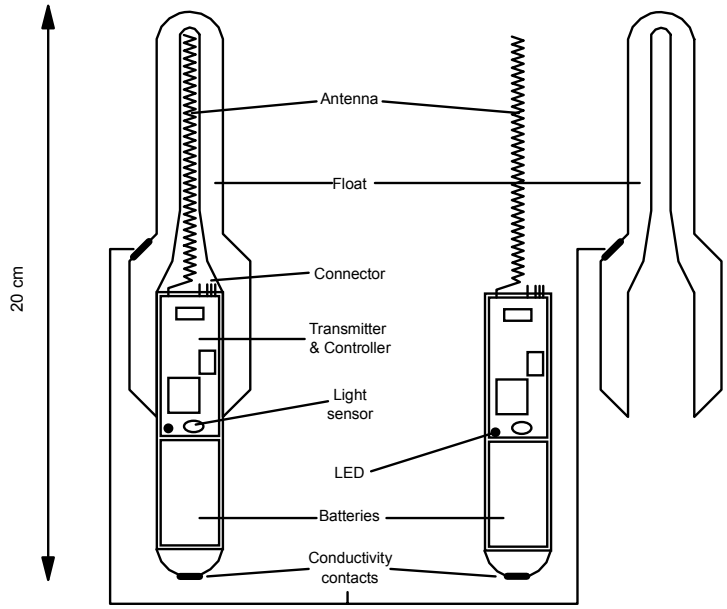


Figure 4. Schematic of standard LHX tag. The device measures 20 cm in length by 5.5 cm in diameter. The positively buoyant tag is fully encapsulated in biocompatible physiological resin.

b. A total of **4** juveniles will be selected annually as controls for the individuals listed in **Task 3.1.a**. These animals will be subject to all handling procedures associated with **Task 3.1.a**, excluding implantation.

Task 3.2. Monitoring of juvenile Steller sea lions with external tracking devices
a. All **16** individuals will undergo the attachment of external monitoring devices for post-release tracking (SLTDR or SPOT tag; dimensions approximately 335 grams, 135 x 35 x 47 mm) via mesh and epoxy.

Task 3.3. Monitoring of stress responses in short-term captive Steller sea lion juveniles
All fecal and blood samples collected under **Task 3** will be analysed for the hormonal and biochemical indicators of stress associated with capture, transportation and short term captivity. In addition, animals selected for life history transmitter implantation will be monitored for stress effects of surgery.
a. Up to **4** juveniles (total of **10** over 5 years) will be physiologically challenged annually with an adrenocorticotropic hormone (Cortrosyn) to determine baseline cortisol responses to a known stressor.
Blood samples will then be spun at 2500 rpm to separate serum. Serum will be pipeted off and stored at -20 degrees C until tested using radioimmunoassay, as described previously by Mashburn and Atkinson (2001). A total of ten animals will be required for this study, as per Gulland et al. 1999. Animals may serve as their own controls (Mashburn and Atkinson 2001). Procedure does not have to occur with all animals at the same time and may take place while animals are undergoing anesthesia for routine health checks.

Task 3.4. Assessment of 3D photogrammetry as a tool for estimating body mass and condition
All data collected via body mass / morphometrics / 3D photogrammetry, ultrasound, will be used to assess and validate the use of 3D photogrammetry as a tool for estimating body mass and condition.
a. Up to **4** juveniles will be selected annually for a brief fasting study (other than those outlined in **Task 3.1**). After an acclimation period in the quarantine environment, these individuals will be subject to:

i. a 2-week fasting period
ii. adipose biopsy pre- and post-fast at 3 sites per animal, on the posterior abdomen, mid-abdomen and neck region (150 mg each)
iii. body composition assessment pre- and post-fast via BIA, ultrasound and isotope dilution

Blubber will be analysed for enzyme activity as described in Mellish et al. (1999), to help determine the mechanisms of fasting physiology. In addition, these samples will be analysed for fatty acid signatures (**Task 3.5**).

Task 3.5. Fatty acid signatures of juvenile Steller sea lions.
Initial blubber biopsies and blood collected under **Task 3** will be aliquoted for fatty acid analysis. Samples collected under **Task 3.4**, including those collected for the brief fasting study, will be analysed for fatty acid profiles. Total lipids will be extracted with 2:1 (vol/vol) chloroform-methanol according to the method of Folch et al. (1957). Butylated hydroxy toluene (Merck) will be added to the extraction solvents at the final concentration of 0.05% to prevent oxidation of polyunsaturated fatty acids (PUFA).

Fatty acids will be converted to methyl esters according to Morrison and Smith (1964) using 14% boron trifluoride by weight in methanol (Sigma). Duplicate analyses of FAME and their identification will be performed using temperature-programmed gas liquid chromatography (GC) on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30m x 0.25 mm id. column coated with 50% cyanopropyl polysiloxane (0.25 film thickness) and linked to a computerized integration system (Turbochrome 6.1 software).

Task 3.6. Vitamin requirements of juvenile Steller sea lions
A subset of the blood and tissue samples collected under **Task 3** (e.g., initial, mid-study, final) will be analysed for vitamin content. Plasma and tissue samples will be frozen at –70°C until analyzed. Plasma will be mixed with methanol and retinyl acetate (internal standard), then extracted with hexane. The hexane layer will be removed, combined, and evaporated under a gentle stream of nitrogen. The residue will be dissolved in 2-propanol. Vitamin A_1, vitamin A_2 (didehydroretinol, and vitamin E (α-tocopherol) will be determined by reversed-phase isocratic high performance liquid chromatography (HPLC). Vitamins will be separated on a Waters C18 Resolve Column (Millipore, Milford, MA) protected with an Upchurch C18 guard column (Upchurch Scientific, Oak Harbor, WA). A mobile phase of methanol/water (98/2) will be used to elute the vitamins. Quantitation for both vitamins will be at 300 nm (Catignani, 1986).

Task 3.7. Metal toxicity in Steller sea lion cell lines
Cells will initially be cultured in commercially available media established for the primary culture of that cell type. All cells will be maintained in a 37°C, humidified incubator with 5% CO_2. Cells lines will be routinely screened for mycoplasma contamination. The process of

immortalization has five steps: infection, selection, assessment, isolation and characterization. Infection assays will be done as described by Bodnar et al. (1998). Cells that have been successfully infected, will be selected by incubating in 400-800 ng/ml puromycin for four days (only those cells with the vector will be able to grow). Surviving cells will be expanded, checked everyday for cell death, and tested regularly for telomerase expression as described below. Monoclonal epithelial cell lines will be selected based on their telomerase expression (Kim et al. 1994, Piatyszek et al. 1995, Holt et al. 1996, Norton et al. 1998). Functionality of the expressed telomerase will be confirmed by measuring telomere lengths. Estimates will be made for the range and median of TRF lengths based on electrophoresis of high molecular weight and 1 kb ladders, respectively (Gibco, Grand Island, New York). Cells expressing the immortalization gene will be seeded at clonal density and a colony derived from a single cell will be ring cloned and expanded into a new cell line. This high expressing cell line will be characterized for karyotype, normal growth parameters (e.g. density-inhibited growth, a normal response to serum deprivation, and anchorage-dependent cell growth), and metal toxicity and compared to uninfected parent cells.

Task 4. Opportunistic utilization of Steller sea lion carcass/tissue sources

This task attempts to maximize the amount of information gathered from opportunistically available Steller sea lion carcasses and portions thereof. Samples not consumed through the following subtasks (4.1, 4.2, 4.3) will be preserved and made available for other researchers through the Alaska Regional Stranding Network.

Task 4.1 Carcass validation of the life history transmitter project

a. We request authorization to implant and deploy up to **30** life history transmitter tags into opportunistically available Steller sea lion carcasses annually. Carcasses will be towed to sea and released in open water, or positioned on beaches, in which case the life history transmitters will be recovered and re-used. The implanted tags will be monitored for a minimum of one year.

Task 4.2. Assessment of reproductive failure

a. Up to **30** expelled placentas will be collected opportunistically annually for examination. Placentas will be examined for basic structure and function, enzymology, contaminants, immunoglobins and endocrinology. They will also be tested for the presence of *Brucella, Leptospira, Chlamydia, Herpes* and other infectious organisms.

b. Up to **30** aborted fetuses will be collected opportunistically annually to be tested for the presence of *Brucella, Leptospira, Chlamydia, Herpes* and other infectious organisms. These fetuses will also be examined for contaminant load.

c. Up to **30** deceased newborns (up to one month of age) will be collected annually opportunistically to be tested for the presence of *Brucella, Leptospira, Chlamydia, Herpes* and other infectious organisms. These neonatal carcasses will also be examined for contaminant load.

d. Major organs (e.g., lungs, kidneys, heart, liver, brain, eyes) of freshly dead animals (up to **30** animals annually) will be collected opportunistically and analysed for contaminant load.

Task 4.3. Metal toxicity in Steller sea lion cell lines

a. Organs of freshly dead animals will be collected opportunistically for cell line development. Cell line development will be achieved through the methods outlined in **Task 3.7**.

Task 5. Development of a floating platform trap method for the capture of Steller sea lions

This task is aimed at the development of an efficient, non-invasive method for the capture and handling of all age classes of Steller sea lions. Platform traps for use at Alaskan sites (e.g., Homer, Seward, Kodiak) would designed in consultation with NMML and similar to their existing traps. These traps employ a 12-ft. wide buoy with a 12-ft. by 12-ft. haul-out platform. Sea lions are typically allowed to haul out and return to the water freely through the trap door. The trap door is dropped for capture purposes when sea lions are hauled out inside. Animals are transferred into a holding cage on 30-foot barge that docks with the capture cage, then moved one at a time from the holding cage into a stainless steel squeeze cage for sampling (i.e., for Tasks mentioned above), followed by immediate release. Design and dimensions of the traps will be based on site-specific characteristics, including other surrounding objects, animal behavior patterns and water depth.

While this task is primarily aimed at the development of this capture technique for future research, we request authorization for the following takes in conjunction with this task:

a. A total of **240** Steller sea lion pups (up to one year, both sexes), **80** juveniles (1-4 years, both sexes) and **80** adults (> 4 years, females) may be captured via the floating trap method.

b. Of those animals captured, **60** pups (both sexes), **20** juveniles (both sexes) and **20** adults (females), may be sampled prior to release, including:

i. gas anesthesia / reversible narcotics
ii. body mass / morphometrics / 3D photography
iii. blood sampling
iv. tissue biopsy (blubber, skin)
v. flipper tag
vi. ultrasound
vii. opportunistic fecal collection
viii. skin and mucosal swabs

Body mass / morphometrics / 3D photogrammetry will be utilized to determine general size and condition of the animal. Blood will be collected to examine the following parameters: hematology, clinical chemistry, viral serology, immuno-competence and stress indicators. Tissue will be analysed for pollutant levels (blubber) and genetics (skin). Flipper tags will be inserted for identification purposes. Ultrasound will be utilized to determine blubber depth, as a measure of overall condition. Fecal samples will be analysed for evidence of parasitic infections and for stress indicators. Skin and musocal swabs will be analysed for epidemiology.

a. *Description of parts or specimen samples*:

Samples taken from live animals during the course of the various projects will include blood, urine, feces, adipose tissue and skin. In addition, non-animate samples will be collected on an opportunistic basis.

Blood. Samples will not exceed 1 cc per kg body mass at time of sampling for all animals to be taken only once. For animals scheduled for multiple sampling, blood volume collected at each take will not exceed the lesser of: a) 1 cc per kg body mass, or b) 5% of total blood volume per month (based on animal mass at the time of collection, as per ASLC protocol, MMPA #881-1443). These samples will be shared amongst Tasks, to be analysed for a full haematology panel, clinical chemistry parameters, as well as hormones, immunology, viral serology and toxicology.

Blubber /adipose tissue. Blubber/adipose tissue (≤ 500 mg) will be collected via biopsy from the neck region, lower caudal region, or alternatively from the incision site of animals undergoing the life history transmitter implantation procedure (**Task 3.1**) to minimize incisions. These samples will be shared among tasks, to be analysed for enzyme activity, hormone levels, fatty acid profiles, pollutant levels and the development of cell lines.

Carcass / placenta / fetus / organ collection. We do not plan to euthanize any animals. However, in the event of opportunistic availability of carcasses or unintentional death, whole carcasses will be collected opportunistically for life history transmitter validation (**Task 4.1**). Whole carcasses of aborted fetuses, newborns (up to one month) and placentas will be collected opportunistically for examination (**Task 4.2**). In addition, major organs (e.g., kidney, liver, brain, lungs, heart and eyes) will be collected for contaminants analysis (**Task 4.2**), and in the event of a very recently deceased animal, cell tissue culture (**Task 4.3**).

Feces. Naturally excreted fecal material will be collected opportunistically from field sites and from short-term captive juveniles. These samples will be shared among tasks to be examined for vitamin content, hormones and evidence of parasitic infection.

Skin. A skin sample (2 x 50 mg) will be collected once from each animal from the flipper via skin punch for genetic analysis and cell tissue cultures. This sample can typically be collected in conjunction with flipper tagging.

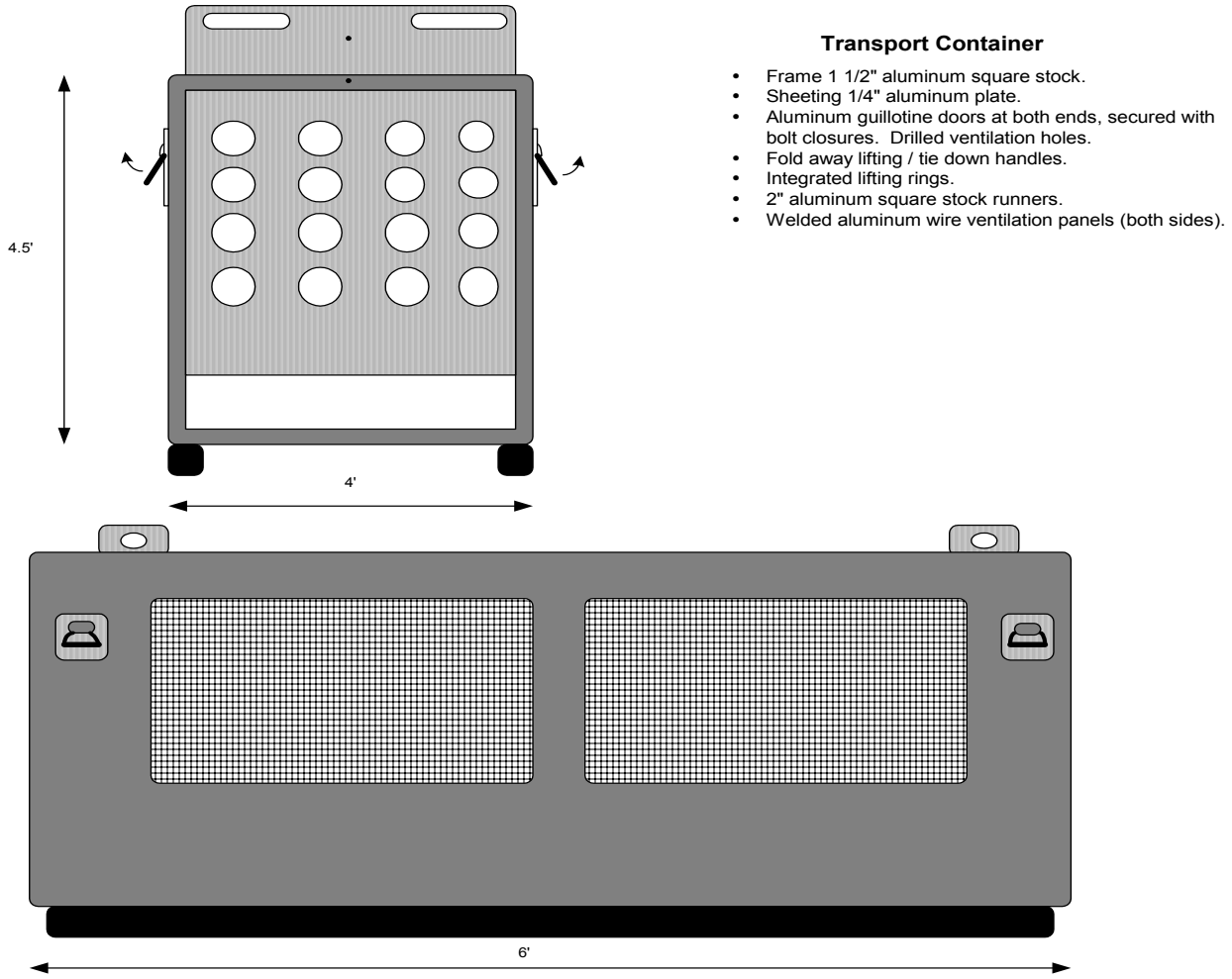
Urine. Urine will be collected opportunistically after voluntary urination or via a catheter and analysed for uric chemistry, sedimentation and bacterial crystals.

b. *Removing animals from the wild/research on captive animals*:
Animals will be removed from the wild for **Task 3**. Given the unfeasibility of multiple recaptures of free-range animals, the utilization of individuals held for observation in short-term captivity will provide the most accurate information possible. Descriptions of all aspects of this task are found in **Sections IV. B**, **IV.B.3** and **IV.C**.

(1) *Explain why a suitable animal(s) cannot be obtained from captive stock*.
In order to collect accurate information to assist the recovery effort for the Steller sea lion stock, the population in question must be monitored and studied. The use of alternate species, captive or free-range, would provide data of limited usefulness. The existing captive Steller sea lion population in North America consists of a few resident individuals at the Alaska SeaLife Center (Alaska), Mystic Aquarium (Connecticut), Oregon Zoo (Oregon) and the Vancouver Aquarium (Canada). There are no currently identified individuals from the Western US stock in long-term captivity. Physiological studies to be conducted during the temporary captive period and behavioral studies post-release are not feasible on long-term captive animals. The utilization of free-ranging animals maintained in captivity for a minimal period of time (i.e., ≤ 3 months) will be essential for accurate data collection. The short-term captive period will allow for sufficient time to conduct research without long-term influences on physiology or behavior. In addition, studies conducted on free-ranging animals will provide essential data pertaining to the stock as it exists in its wild state, to compare with possible alterations or influences of long-term or short-term captivity.

(2) *Provide a description of the enclosure to be used for containment and the transport, mode of transportation, special care during transport, and the length of time required for the transfer from the capture site to the initial holding facility, and then to the permanent holding facility*. Juvenile Steller sea lions (1-4 years of age) will be captured in water or on land using standard techniques developed by Don Calkins and Dennis McAllister, as described in **Section IV.C**. Animals will be transported from the site via ship or helicopter within a 24 hour period (e.g., **Figure 5**). For non-immediate transfer via support vessel (i.e., transport time of up to 48hrs), animals will be held in specialized holding containers A veterinarian will be present at the departure location to ensure that the animal is in good condition and can withstand transport. A transport container similar to those currently in use by ADF&G and NMML (e.g., dimensions approximately 69" long x 15" high x 24" wide) will be used that will confine the animal but not entirely restrict movements. Generally, chemical restraint will be not be used. No animal will be transported in an agitated state. All animals will be allowed to acclimate to the transport container.

Figure 5. Schematic of juvenile transport container.



(3) *If the source stock is to be beached/stranded marine mammals, indicate the name and location of the rehabilitation facility.*
Not applicable.

(4) *If the source stock is from marine mammals already in captivity (other than beached/stranded animals) indicate the name and location of the facility, and identify the specific animals involved in the proposed activity*.
Not applicable.

(5) Include a copy of any license or registration issued by the Animal and Plant Health Inspection Service of the U.S. Department of Agriculture, any outstanding variances granted by APHIS, and the most recent APHIS inspection report.
Copies of the most recent APHIS certificate and report are included in **Additional Documents**.

(6) *Include the comments and recommendations of any relevant Institutional Animal Care and Use Committee established under the Animal Welfare Act (AWA) (7 U.S.C. 2131 et seq.)*
Each investigator is required to have approved assurances / animal care protocols to both the Alaska SeaLife Center and their respective institution prior to initiation of research activities.

(7) *Provide a written statement from the responsible veterinarian certifying that the facilities, methods of care and maintenance, and methods of transport will be adequate to ensure the well-being of the marine mammals and comply with all applicable care and transport standards established under the AWA.*
A copy of this statement is included in **Additional Documents**.

(8) *If release of captive marine mammals to the wild is proposed, state the length of time the animals will be held, and describe the protocols for the release addressing mitigation measures for the following concerns*:
-disease transmission between both released animals and the wild population;
Each group of captive transient animals (**Task 3**) will be transported and maintained at the ASLC for periods of up to 3 months. Individuals will be maintained in a specially designated quarantine area (**Figures 6 & 7**) to eliminate any potential disease transmission between the resident and transient individuals.
These groups (maximum 4 animals per group) will be held in one of two quarantine enclosures:

Quarantine 1 (2002-2003)
This enclosure utilizes existing quarantine facilities at the Alaska SeaLife Center (**Figure 6**). It includes two outdoor tanks, one 20' diameter pool and a second 12' diameter pool. This area is completely separated from the remainder of the outdoor facilities by a 10' concrete wall and two sets of watertight doors. Therefore, individuals will be physically and visually removed from the rest of the facility. Food preparation and support for the quarantine animals will occur in a quarantine corridor, that is, a pathway fenced off to all other areas and accessible only by persons involved with the transient program. Whenever possible, blinds will be used during maintenance and feeding events to reduce habituation to human presence.

Quarantine 2 (2003-2006)
This enclosure is currently in the planning stages and will be constructed in 2002, as fully contained unit separate from the ASLC main building (**Figure 7**). It has been specifically designed and designated for the transient juvenile Steller sea lion program. It will consist of four 12' diameter pools, separated by a 10' corridor. The inner structure will be surrounded by 10' chain link fence. A complete food preparation and support area will be included in this structure, with an observation area. As in Quarantine 1 (the currently existing structure at the ASLC), transients will only be housed with other transients, and will be physically and visually removed from all other animals. Whenever possible, blinds will be used during maintenance and feeding events to reduce habituation to human presence.

Quarantine Procedures
Much is still to be learned about contagious diseases in marine animals, particularly Steller Sea Lions. The general principles of quarantine and disease control will be essential to the Transient SSL Program and utilized at all times to prevent possible spread of disease between 1) individual wild caught animals; 2) wild caught animals and other animals at ASLC (collection, research and rehabilitation populations); and 3) humans and domestic animals and all of the above. Because diseases can be transmitted in many and often inapparent ways and strict adherence to the isolation protocols will be difficult within the facility, all personnel entering or working in the juvenile Steller sea lion quarantine holding area or with juvenile Steller sea lion capture and field work components must be well versed in the principles and practice of disease control, animal isolation and quarantine procedures. This will require certification by ASLC veterinary services through class attendance or substitution of equivalent training and experience. Individuals, certified or otherwise, not directly necessary to the husbandry and ongoing research procedures may not enter the security/animal enclosure door. Non-certified individuals may only enter the primary animal holding area if accompanied by a regular juvenile Steller sea lion staff member who will be responsible for that person's adherence to quarantine policy.

Each group of juvenile Steller sea lions will be handled as distinct and separate risks. All surfaces (decks, pools, walls, doors, faucets & drains, food containers, restraint devices carts, etc.) of the quarantine holding area will be thoroughly cleaned of all particulate and organic matter with appropriate detergent and scrubbing, then disinfected with steam or bleach (sodium hypochlorite) for appropriate contact time (20-30 minutes). Surfaces should then be allowed to air dry (for at least 24 hrs.) and dissipate residual chlorine before introduction of new arrivals.

Inner and outer quarantine spaces will be designated as red zones and yellow zones depending on use and presence of animals or known disease. The primary animal holding area will be considered a red zone at all times. The loading dock entry and walkway and pathways up to and including storage areas for capture and research equipment will also be considered red zones 1) until fully disinfected after arrival of juvenile Steller sea lions; 2) whenever marine mammals are present in rehabilitation; 3) if any suspect illness is noted in any animal within ASLC (outdoor holding, rehabilitation or collection research animals).

Red Zone
- foot baths (and when necessary, vehicle / cart wheel sponges) containing dilute chlorine bleach (1oz/gal) will be placed at all entrance ways and maintained at a depth no less than 1/2". Personnel must step into the footbaths and push/drive all wheels across the freshly saturated sponge when entering and exiting.

- when entering the animal holding area, personnel must change into footwear provided (rubber boots) and use secondary foot baths when entering or exiting the animal enclosure.
- disposable or reusable (raingear) protective pants and tops will be donned on entrance to animal holding areas or whenever handling animals. This clothing must be thoroughly disinfected after each use by spraying and washing all outer surfaces with dilute chlorine or betadine solution, and then hung to dry in the changing area. Reusable gear will be laundered and disinfected at least two times per week.
- rubber or vinyl gloves must be worn whenever working in animal enclosures or handling animals. These should be disposed of in designated waste containers before exiting changing area. Hands must be washed thoroughly with disinfectant soap and rinsed after removing gloves and protective outerwear and as often as possible when working in red or yellow zones.
- food containers used in the quarantine holding must not leave the area. Food should be delivered to appropriate refrigerated storage (cooler or refrigerator) in disposable plastic bags each morning and bags and left over food disposed of within the quarantine holding area no more than 24 hours after delivery.
- after entering the quarantine holding, personnel are restricted from entering <u>any</u> other area of the ASLC (food kitchen or freezer, research corridor, loading dock/receiving areas or marine mammal exhibit or housing areas) until they have showered and exchanged all outer clothing.
- any equipment used inside an animal enclosure or in direct contact with a juvenile Steller sea lion (restraint devices, feeding utensils, research equipment, etc.) must be washed, rinsed and treated with disinfectant (dilute bleach, chlorhexidine or povidine iodine) prior to storage or use on another animal.
- personnel cleaning with hoses or pressure washers must avoid sprays that lift droplets into the air at heights that might allow transit over the dividing walls.

Yellow Zone
- dual use or access areas to the primary juvenile Steller sea lion quarantine holding enclosure will be thoroughly cleaned and disinfected (see above) after exposure by presence or transit of a marine mammal. After this disinfection process, these areas will be considered yellow zones i.e.: loading dock, access hallway to outdoor laboratories, necropsy room veterinary clinic and quarantine corridor.
- while in yellow zone condition personnel may enter these areas without protective clothing but must still use footbaths and equipment wheel sponges and sprays and must change into boot covers or quarantine boots if proceeding into primary animal housing areas with no exceptions.
- all nonessential traffic in these areas is prohibited.
- access to outdoor laboratories via the outdoor loading dock and corridor (e.g., movement of large equipment) must be scheduled with juvenile Steller Sea Lion Project manager so the corridor can be properly cleaned and disinfected before and after transit.

General
- only dedicated juvenile Steller sea lion equipment (PPE, capture and restraint devices, food and waste containers, research equipment, etc.) may be brought into the quarantine holding area. Likewise, none of this equipment should leave the quarantine holding area until completely disinfected. Trash, including food waste, should be bagged inside the primary areas and a second bag properly applied during exit from the area, and then disposed of directly into the dumpster.

- hazardous waste (sharps, blood contaminated or containing potentially infected material) should be contained in appropriate red bags and designated boxes until pickup by contractor for incineration.
- no human food or drink will be allowed in the red or yellow zones.
- no other animals will be allowed in the red zone. Personnel should avoid direct contact with other animals prior to working in red or yellow zones and wash well or change outer clothing as needed (Pet hair and saliva, salvage of carcasses or other animal parts, processing of blood, feces or other animal tissue, etc.).
- personnel should report any suspected illness to their supervisor and avoid coming into contact with animals, their food or other direct contact equipment until fully recovered.
- individuals should assist each other in donning protective equipment and maintaining proper vigilance for possible breaks in quarantine procedure. Breaks in quarantine should be reported to the appropriate supervisor and to Veterinary Services for appropriate cleanup and response.
- lock-out/tag-out procedures must be fully utilized to prevent backflow of contaminated water into other outdoor laboratories.

Cleaning Procedures

Disinfectants are to be used to clean pen and feed areas or as external antiseptics to clean wounds or prepare surgical sites. Care must be taken to properly dilute the solution required. Contact of disinfectants to skin should be avoided; make sure to rinse pens, pools and animals areas thoroughly. The type or disinfectant is rotated on a monthly basis due to the fact that pathogens may become resistant to a type of disinfectant over periods of time. The disinfectant of the month will be indicated by a sign. Do not mix disinfectants or overlap on surfaces. Some combinations can produce toxic gases or may inactivate the ingredients. Most disinfectants lose potency rapidly on exposure to exterior light. Fresh dilutions should be prepared daily.
- *Sodium hypochlorite*: household bleach (2 oz. in 1 gallon of water). Effective against bacteria, viruses, and fungi. Effective in the presence of soap and hard water. pH is acidic. Corrosive, may damage metal surfaces. Ensure adequate ventilation is present before using.
- *Roccal-D*: quaternary ammonia (1 oz. in 1 gallon of water). Effective against bacteria and fungi. Effective in the presence of hard water. pH is alkaline.
- *Betadine*: iodophore (2 oz. in 1 gallon of water). Effective against bacteria, virus, fungi and spores. Effective in the presence of soap and hard water. The pH is neutral. Safe on tissues.
- *Nolvasan*: Chlorihexidene (solution and scrub; 1 oz. in 1 gallon of water). Effective against bacteria, fungi and viruses. pH is alkaline. Safe on tissues when diluted.

Figure 6. Schematic of 2002-2003 juvenile quarantine environment.

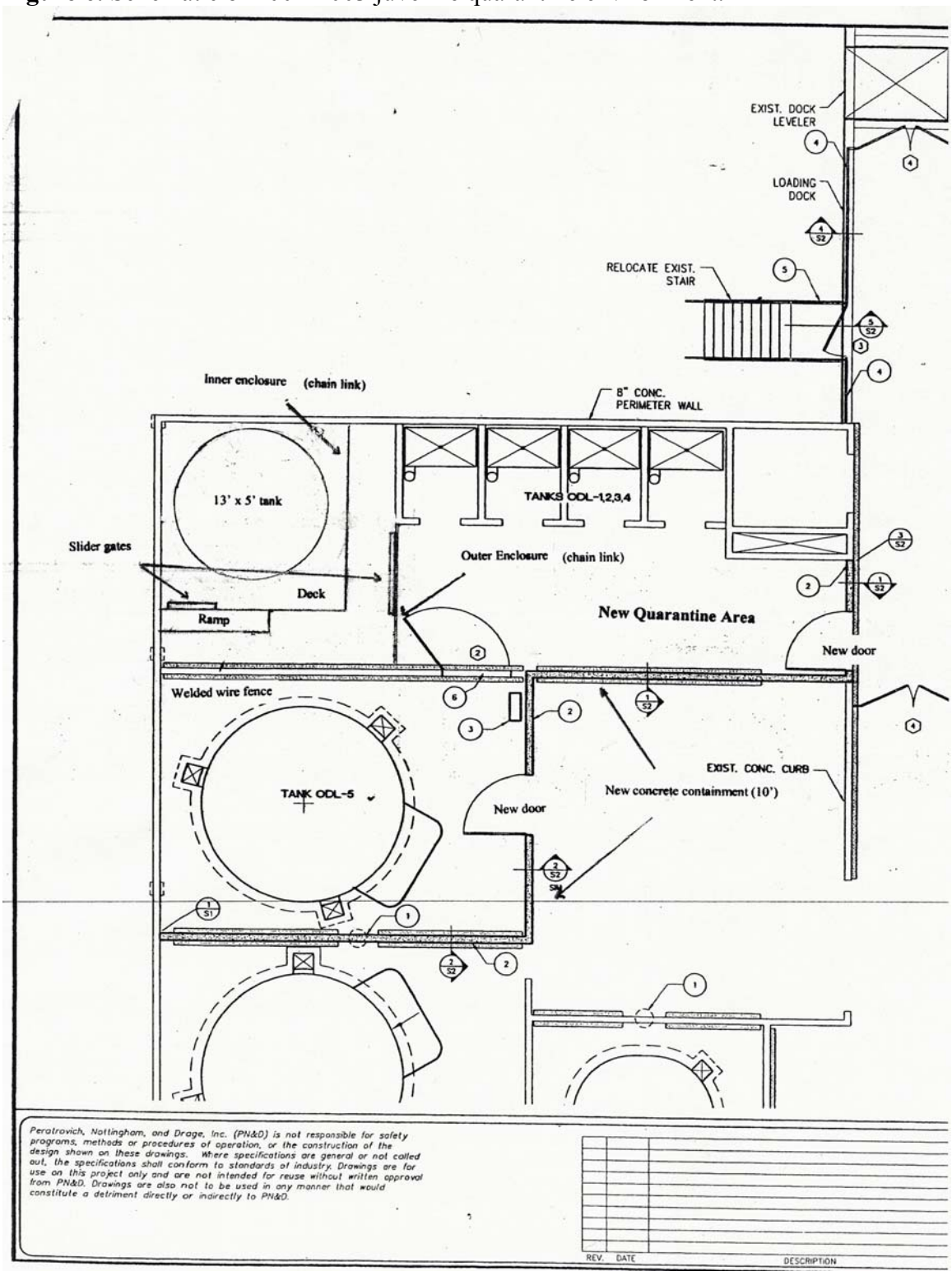
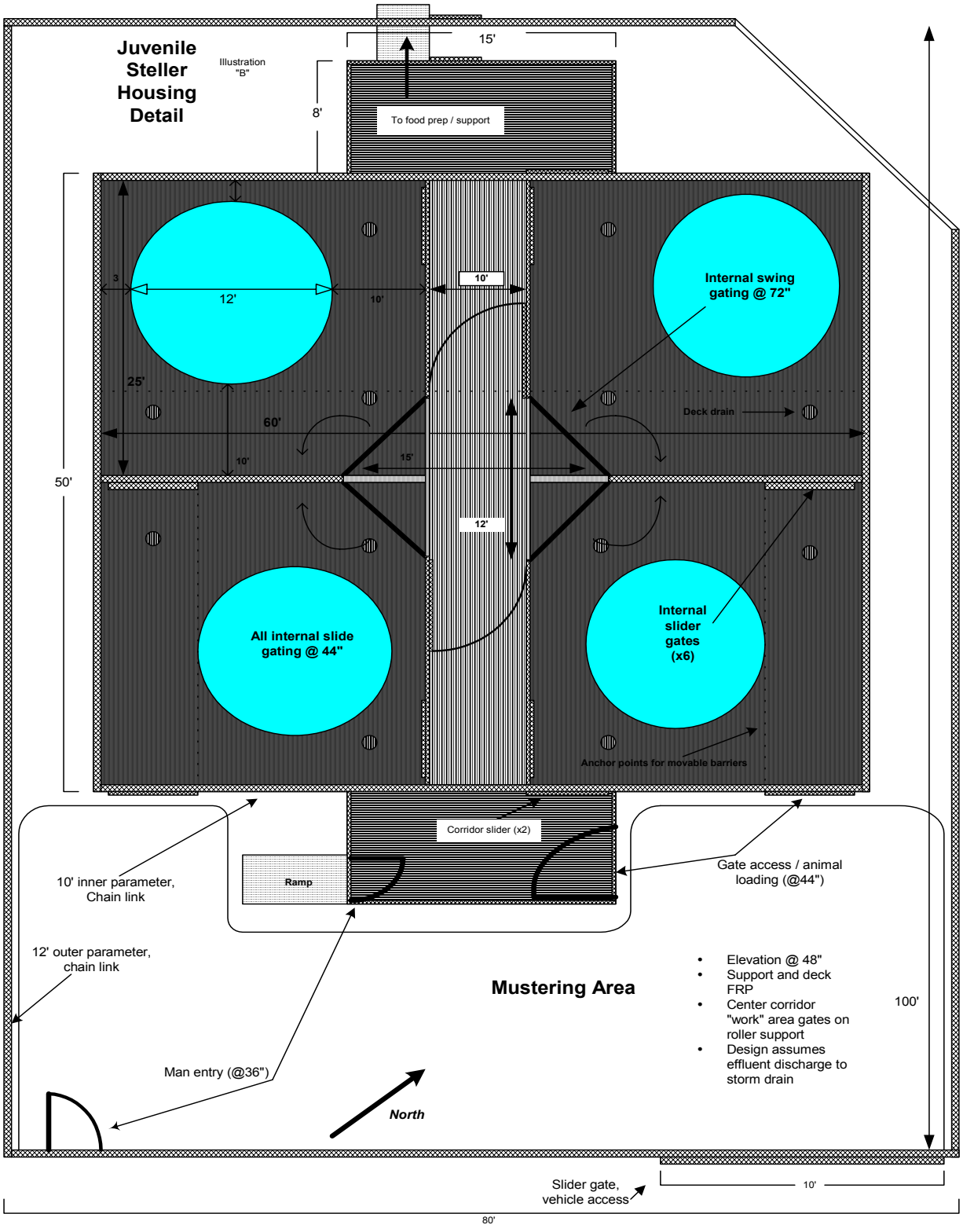


Figure 7. Schematic of 2003-2006 juvenile quarantine environment.



All transient individuals will undergo a comprehensive health screening at the time of capture and again prior to release.

Health Screening

Our proposed approach to health screening is outlined below. This list of parameters has been compiled with the assistance of multiple veterinarians familiar with marine mammal practices. This protocol may be expanded and/or modified pursuant to further input. Parameters to be assessed and examined by the attending veterinarian are:

1. body condition
   - mass
   - morphometrics
   - isotope dilution / BIA
   - ultrasound for blubber depth
2. haematology
3. clinical chemistry
4. viral serology / bacteriology (*Leptospirosis, Chlamydia, Herpes, Poxvirus, Papilloma, Adenovirus, Morbillivirus, Brucella, Pastuerellosis, Mycobacterium*)
5. stress indicators (thyroid hormones)
6. tissue organochlorine/toxin load
7. fecal collection (parasitic infection, stress response)
8. skin / mucosal swabs (epidemiology)
9. urinalysis (metabolites)
10. X-ray (congenital deformities, broken bones)

- *unwanted genetic exchanges between introduced and endemic stocks*;

Transients will be released only in the immediate region of their original location, eliminating any artificial mixing of genetic stocks.

- *ability of the released animals to forage and protect themselves from predators;*

Only weaned animals greater than one year of age will be selected for the transient program (**Task 3**). Field tests are under way to determine physiologically if an animal is still suckling but have not been developed yet. Currently we can tell if an animal is weaned buy looking at size and eruption and wear patterns in the teeth. Larger animals (larger than 100 kg) and animals that have canine teeth longer than teeth on either side are considered older than 1 year and weaned. Therefore, transients will have developed adequate foraging and predator avoidance behaviors prior to capture. All efforts will be taken to minimize exposure to humans, including visual quarantine of the animals. Whenever possible, blinds will be used to prevent habituation. Also, the use of live prey, and/or a mix of live and dead prey will be utilized whenever feasible. The brief period of holding at the ASLC should minimize any loss of these behaviors. No relevant information currently exists to evaluate the effect of short-term captivity on an individual's ability to avoid predators compared to their cohorts.

- *elimination of behavioral patterns acquired during captivity that could prove detrimental to the released animals or the social structure of local populations*.

Short-term captive transient juveniles (**Task 3**) will only be housed with other transients, eliminating any learned behavior from the resident, captive animals. In addition, the transient

juveniles will be visually quarantined from the long-term captive Steller sea lions as well as from human presence to the greatest extent possible. The limited time period the animals are to be held will minimize any alterations in their normal or pre-existing behaviors.

c. *Import/Export of Marine Mammals/Marine Mammal Parts: Describe the import/export of marine mammals or parts, including the country of exportation and the country of origin.*
There are no current requests to import/export marine mammal parts in conjunction with this permit application.

d. *Lethal take***:**
Field studies - There is no intentional lethal take in conjunction with any aspect of this application. However, we request authorization for the unintentional mortality of up to 5 animals per year during field procedures, with a five-year maximum of 15 mortalities for the field component of the program (see **Table 1**).
At the ASLC - In addition, we request the authorization for the unintentional mortality and/or euthanasia of up to 3 animals per year associated with the captive transient program at the ASLC. At the onset of the program, the team of Investigators, Co-Investigators and Veterinarians involved will decide upon a panel of health criteria for the participation of an animal in the captive, transient program. In the event that an animal is deemed non-releasable at the final examination, we request authorization for the following options: 1) extension of the short-term period of captivity at the ASLC for additional veterinary care; 2) maintenance of the animal in long-term captivity at the ASLC; 3) identification of another location for long-term captivity; or 4) euthanasia. If more than 3 animals are deemed non-releasable within the period of one year, we will review and re-evaluate the process.

4. Publication of Results:
Research results will be published and made available in the appropriate refereed scientific journals at the discretion of the ASLC researchers and faculty and the investigators involved with the respective research programs to be implemented. All publications will be in compliance with any funding requirements.

D. National Environmental Policy Act (NEPA) Considerations

(a) does the research involve new, innovative, controversial or experimental equipment or techniques;
The majority of the research tasks outlined within this application have been performed in part or in whole previously with Steller sea lions or with other marine species. The application of the life history transmitter project to Steller sea lions is the first for this species, therefore, this project may be considered new and innovative research. However, similar instruments have been used successfully in other marine species, such as the sea otter (Ralls & Siniff 1990, Ralls et al 1989, Siniff 1985, Siniff & Ralls 1991, Monnett & Rotterman 2000, Garshelis & Siniff 1983, Williams & Siniff 1983) and polar bear (Mulcahy & Garner 1999).

(b) are the research techniques likely to be adopted by other researchers;
Yes. Several procedures outlined in this permit application have previously and are currently utilized with Steller sea lions and other species.

(c) is the location in which the research will be conducted of special importance;
This research will take place throughout the Alaskan range of the Steller sea lion. Similar research has been routinely conducted in this area for several years. Continuing research efforts will also be conducted on the Russian population of the Steller sea lion in collaboration with Russian investigators.

(d) do the proposed activities involve unique or unknown risks or are the effects highly uncertain;
Most activities outlined in this application involve widely-used procedures (e.g., capture, anesthesia, blood sampling, biopsy, tagging) and do not have previously unidentified risks. The life history transmitter project involves a procedure of surgical implantation, which will be the first of its kind for this species. Any project involving surgery that has not been practiced as such on this species involves and uncertain risk which cannot be quantified at this time. However, this and similar procedures have been used successfully for many years in other aquatic and terrestrial mammals, and we have made all attempts to develop procedures that minimize predictable risks. In the event that a new or unidentified risk is identified, procedures will be revisited and revised to the best interests of the animal.

(e) *will any aspect of the research possibly affect the public health or safety of humans*;
No. All contact with free-ranging and temporarily captive Steller sea lions will take place with standard safety procedures. All animals involved in the captive transient program and associated sub-Tasks will be subject to strict quarantine protocols.

(f) *will the activity have a significant cumulative effect, considering existing and potential activities*;
All measures to minimize any disturbance or negative impact on the free-ranging and captive populations have been carefully considered and included when feasible. For instance, this includes multiple projects utilizing a single sample (i.e., reduce the number of sampling events). To the best of our knowledge, this application will provide the maximum amount of data with

the minimum amount of impact to the population. We are unaware of other current studies that may negatively complicate the impact of our proposal.

(g) *will the activity cause loss or destruction of significant scientific, cultural, or historic resources;*
No.

(h) *will there be an adverse effect on endangered or threatened populations or stocks or their habitat;*
No. The activities of this permit application are directed towards the recovery effort of an endangered species and all efforts will be made to minimize disturbance.

(i) *is the activity in violation of a Federal, State, or local law for environmental protection;*
No.

V. Previous and other permits

A. Previous permits: Not applicable.

B. Other permits:
National Marine Fisheries Service, Office of Protected Resources (#881-1443)
US Department of Agriculture, Animal and Plant Health Inspection Service (#96-R-0005)
US Department of the Interior, Fish and Wildlife Service (#01-015)

VI. Special considerations for Applicants working abroad (for exports of parts/samples or live animals from the US).

There are no requests to export live animals or animal parts from the US at this time.

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VIII. Certification and Signature

"I hereby certify that the foregoing information is complete, true, and correct to the best of my knowledge and belief. I understand that this information is submitted for the purpose of obtaining a permit under one or more of the following statutes and regulations promulgated thereunder, as indicated in the Section I. of this application:
The Endangered Species Act of 1973 (16 U.S.C. 1531-1543) and regulations (50 CFR 222.23(b)); and/or
The Marine Mammals Protection Act of 1972 (16 U.S.C. 1361-1407) and regulations (50 CFR Part 216); and/or
The Fur Seal Act of 1966 (16 U.S.C. 1151-1175).
I also understand that any false statement will subject me to criminal penalties of 18 U.S.C. 1001, or to penalties provided under the Endangered Species Act of 1973, the Marine Mammal Protection Act of 1972, or the Fur Seal Act of 1966, whichever are applicable."

Signature of Applicant _____

Date _____

Typed name of Applicant Tylan Schrock

Title of Applicant Executive Director

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Education

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1972	M.S.- University of Alaska, Fairbanks (Wildlife Management)

Professional Experience

2001 - present	Steller Sea Lion Program Manager, Alaska SeaLife Center
1998-2000	Senior Marine Mammal Scientist, Alaska SeaLife Center, coordinator of Steller Sea lion Research
1998 - present	Consultant, Calkins Wildlife Consulting
1993	Supervisor, Alaska Department of Fish and Game
1992-1997	Principal Investigator, Alaska Department of Fish and Game Steller sea lion research
1991- present	Member, Steller Sea Lion Recovery Team
1989-1991	Oil spill studies coordinator, Alaska Department of Fish and Game, Principal Investigator, Steller sea lion oil spill studies
1988	Staff Biologist, Alaska Department Fish and Game
1974-1987	Marine Mammals Biologist, Alaska Department of Fish and game extensive work on Steller sea lions, harbor seals sea otters and Beluga whales, Principal Investigator, Steller sea lion research
1973-1974	Game Biologist, Alaska Department of Fish and Game

Selected Publications

2000 Pitcher, K. W., **D. G. Calkins** and G. W. Pendleton. Steller sea lion body condition indices. Marine Mammal Science. 16;427-436

1999 Saeki, K., Nakajima, M., Noda, K., Loughlin, T. R., Baba, N., Kiyota, M., Tatsukawa, R. and **D. G. Calkins**. Vanadium accumulation in pinnipeds. Archives of environmental contamination and toxicology.

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Publications

Mellish JE and SJ Iverson (2001) Blood metabolites as indicators of nutrient utilization in fasting, lactating phocid seals: does depletion of nutrient reserves terminate lactation? Canadian Journal of Zoology 79: 303-311.

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Associate Researcher, Hawaii Institute of Marine Biology, University of Hawaii 1991- 2000
Affiliate Researcher, Hawaii Institute of Marine Biology, University of Hawaii 1989-1991
Experimental Scientist, Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Animal Production, Western Australia

Selected Publications
Atkinson, S and Gilmartin, WG. (1992) Seasonal testosterone pattern in Hawaiian monk seals. J. Reprod. Fert. 96:35-39.

Atkinson, S, Gilmartin, WG and Lasley, BL. (1993) Testosterone reduction after injection of gonadotropin releasing hormone in male Hawaiian monk seals. J. Reprod. Fert. 97:35-38.

Atkinson, S, Becker, BL, Johanos, TC, Pietraszek, JR and Kuhn, BCS. (1994) Reproductive status and morphology of female Hawaiian monk seals that were fatally injured by male seals. J. Reprod. Fert. 100:225-230.

Pietraszek, JR and **Atkinson, S**. (1994) Concentrations of oestrone sulfate and progesterone in plasma and saliva, vaginal cytology, and bioelectric impedance during the oestrous cycle of the Hawaiian monk seal. Mar. Mamm. Sci. 10:430-441.

Iwasa, M and **Atkinson, S**. (1996) Analysis of corpora lutea to estimate reproductive cycles of wild Hawaiian monk seals (*Monachus schauinslandi*). Mar. Mamm. Sci. 12: 182-198.

Iwasa, M, **Atkinson, S** and Kamiya, S. (1997) Lipofuscin granular cells in regressing corpora lutea and corpora albicantia of ovaries from wild Hawaiian monk seals. Mar. Mamm. Sci. 13:326-332.

Goodman-Lowe, G, **Atkinson, S**, and Carpenter, JR. (1997) Initial defecation time and rate of passage of digesta in adult Hawaiian monk seals. Can. J. Zool. 75:433-438.

Atkinson, S. (1997) Reproductive biology of seals. Reviews of Reproduction. 2:175-194.

Silvers, LE, **Atkinson, S**, Iwasa, M, Combelles, C and Salden, D. (1997) A large placenta encountered in the Hawaiian winter grounds of the humpback whale, *Megaptera novaeangliae.* Mar. Mamm. Sci. 14: 175-180.

Mazzuca, L, **Atkinson, S** and Nitta, E. (1998) Deaths and entanglements of humpback whales in the main Hawaiian Islands, 1973-1995. Pac. Sci. 52:1-13.

Theodorou, J. and **Atkinson, S**. (1998) Monitoring of total androgen in saliva from captive Hawaiian monk seals. Mar. Mamm. Sci. 14: 304-310.

Crow, GL, Atkinson, MJ, Ron, B, **Atkinson, S**, Skillman, DK, and Wong, TF. (1998) Relationship of water chemistry to serum thyroid hormones in captive sharks with goitres. Aquatic Chemistry. 4:469-480.

Atkinson, S, Ragen, T, Gilmartin, WG, Becker, BL and Johanos, TC. (1998) Use of a GnRH agonist to suppress testosterone in wild Hawaiian monk seals. Gen. Comp. Endo. 112:178-182.

Goodman-Lowe, GD, Carpenter, JR and **Atkinson, S**. (1999) Assimilation efficiency of prey in the Hawaiian monk seal, *Monachus schauinslandi*. Can. J. Zool. 77:653-660.

Mazzuca, L, **Atkinson, S**, Keating, B and Nitta, E. Mass strandings of cetaceans in the Hawaiian Archipelago, 1957-1995. Aquatic Mammals. 25:105-114.

Atkinson, S, Combelles, C, Vincent, D, Nachtigall, P, Pawloski, J, and Breese, M. (1999) Monitoring of progesterone in captive female false killer whales, *Pseudorca crassidens*. Gen. Comp. Endo. 115:323-332.

Crow, GL, Ron, B, **Atkinson, S** and Rasmussen, LEL. Serum T4 and T3 concentrations in immature whitetip reef sharks, *Triaenodon obesus*. J Exp. Zool. 284:500-504.

Goodman-Lowe, GD, Carpenter, JR, **Atkinson, S**, and Ako, H. Nutrient, fatty acid, amino acid and mineral analysis of natural prey of the Hawaiian monk seal, *Monachus schauinslandi.* Comp. Biochem. Physiol. Part A 123:137-146.

West, KL, **Atkinson, S**, Carmichael, MS, Sweeney, JC, Krames, B, and Krames, J. Progesterone concentration in bottlenose dolphin milk in relation to reproductive status. Gen. Comp. Endo. 2000. Feb, vol. 117.

Atkinson, S. (2000) Novel Approaches to endocrinologic monitoring in marine mammals. Rep. Bottlenose Dolphin Repro. Workshop San Diego, CA June 3-6 1999

Feinholz, DM, and **Atkinson, S**. (2001) Possible etiologies of yellow coloration in dolphin calves. Aqua. Mamm. 26: 191-195.

Robeck, TR, **Atkinson, S**, and Brook F. (2001) Reproduction. *In* CRC Handbook of Marine Mammal Medicine. 2nd Edition. Dierauf , LA and Gulland, FMD. Eds. CRC Press pg. 193-236.

Atkinson, S. (2001) Male reproductive systems*. In* Encyclopedia of Marine Mammals. Academic Press. In Press.

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Education

Doctor of Veterinary Medicine (Honours) 1970
Washington State University-Pullman, Washington

Professional Experience

Veterinarian, Alaska SeaLife Center - Seward, Alaska 1997- present

Practicing Veterinarian, College Village Animal Clinic 1971- present
Anchorage, Alaska

Veterinary Consultant 1995 - present
- U.S.Fish and Wildlife Service
  - Sea Otter Abdominal Transmitter Implants - Cordova, Alaska
  - Walrus Research Project - Cape Peirce, Alaska
  - Common Murre Transmitter Implants - Homer, Alaska
  - Brandt Geese Heart Rate Transmitter Implants- Anchorage, Alaska
  - Sea Otter Pup Nursery Care and International Transport
- National Marine Fisheries Service
  - Juvenile Steller Sea Lion Field Anesthesia
- Canadian Wildlife Service, North Slope Borough, US Fish and Wildlife Service
  - King, Spectacled and Common Eider Satellite Transmitter Implants

Professional Associations/Honors

American Veterinary Medical Association	1970 - present
Alaska State Veterinary Medical Association	1970 - present
President	1974 - 1975
Chairman - Peer Review Committee	1988 - 1995
American Animal Hospital Association	1974 - present
Regional Practitioner of the Year	1989
Association of Avian Veterinarians	1982 - present
State Member Representative	1992 - present
International Association of Aquatic Animal Medicine	1990 - present
American Association of Wildlife Veterinarians	1997 - present
American Association of Zoo Veterinarians	1998 - present

Selected Publications

Tuomi, P., 2001, "Sea Otters" in: Handbook of Marine Mammal Medicine, Dierauf, L.A. and F.M.D. Gulland, eds., CRC Press, Baca Raton, FL pg. 961-988.

Tuomi, P., M. Grey, and D. Christen, 2000, "Butorphanol and Butorphanol/ Diazepam Administration for Analgesia and Sedation of Harbor Seals (*Phoca vitulina*)", in: Proceedings of American Association of Zoo Veterinarians and International. Association of Aquatic Animal Medicine Joint Conference, Baer, C.K. and R.A. Patterson, eds., New Orleans, LA , pg 382-383.

Tuomi, P., and T. Williams, 1995, "Effects of Oiling and Rehabilitation on Pregnant and Newborn Sea Otters", in: Proceedings of IWRC Oiled Wildlife Symposium, Seattle, Wa., Rineer-Garber, C., ed., pg.218-221.

Tuomi, P., D.M. Mulcahy and G.W Garner, 1996, "Immobilization of Pacific Walrus (*Odobenus rosmarus divergens)* with carfentinil Reversal and isoflurane anesthesia" in: Proceedings of International Association for Aquatic Animal Medicine, Abt, D.A., ed., Chattanooga, TN., Vol. 27, pg. 121-123.

Tuomi, P., S. Donoghue, J. Otten-Stranger, 1995. "Husbandry and Nutrition", in: Emergency Care and Rehabilitation of Oiled Sea Otters: A Guide for Oil Spills Involving Fur-bearing Marine Mammals, Williams, T.M. and R.W.Davis, eds., University of Ak. Press, Fairbanks, Ak., pg. 103-120.

Williams, T., R. Davis, J. McBain, **P. Tuomi**, R. Wilson, C. McCormick, and S. Donoghue, 1995. "Diagnosing and Treating Common Clinical Disorders In Oiled Sea Otters". Ibid, pg. 59-94.

Tuomi, P., 1990, "Husbandry - Valdez Rehabilitation Center", in: Bayha, K. and J. Kormendy, Tech. coord., Proceedings of the Sea Otter Symposium Following the T/V Exxon Valdez Oil Spill, Anchorage, Alaska, April 17-19, 1990, USFWS Biol. Rep. 90(12), pg. 274-284.

Harris, R.K., Moeller, R.B., Lipscomb, T.P., Haebler, R.J., **Tuomi, P.A**., McCormick, C.A., DeGange, A.R., Mulcahey, D., Williams, T.D., and Pletcher, J.M., 1990, "Identification of a herpes-like virus in sea otters during rehabilitation after the T/V Exxon Valdez oil spill." In: Sea Otter Symposium: Proceedings of a symposium to evaluate the response effort on behalf of sea otters after the T/V Exxon Valdez oil spill into Prince William Sound, Anchorage, AK 17-19 April 1990. K. Bayha and J. Kormendy, (eds), U.S. Fish and Wildlife Service Biological Report, 90,12: 366-368.

Tuomi, P., 1990, "Rehabilitation Notes: Sea Otter Pups (*Enhydra lutris*)", Wildlife Journal, International Wildlife Council, Vol. 13, No. 9, pg 9-14.

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Education

1993-1997: Doctor of Veterinary Medicine.
Auburn University, College of Veterinary Medicine

1989-1993: B.S. in Zoology, minor in Marine Biology.
Auburn University

Professional Experience

Marine Animal Veterinarian, Alaska SeaLife Center, Seward, AK	present
Emergency and Critical Care Veterinarian Alameda East Veterinary Hospital, Denver, CO	3/00-9/00
Emergency and Critical Care Veterinarian Animal ER of San Diego, San Diego, CA	9/99-2/00
Chief of Staff Veterinarian Vetsmart Pet Hospital, Encinitas, CA	11/98-9/99
Small Animal, Avian and Exotic Veterinarian Medical Management Inc. Vetsmart Pet Hospital, San Diego, CA	6/97-11/98
Zoo and Wildlife Veterinary Preceptor Bronx Zoo, Bronx, NY	5/97-6/97
Marine Animal Preceptor Sea World of California, San Diego, CA	4/97-5/97
Zoo Animal Veterinary Intern Marine World, Africa USA, Vallejo, CA	3/97-4/97
Wildlife Veterinary Intern Wildlife Safari, Winston, OR	1/97-3/97
Surgical Veterinary Assistant Auburn University, Auburn, AL	11/96-1/97
Dallas Zoo and Aquarium Intern Dallas Zoo, Dallas, TX	5/95-9/95

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Education

1972-1977	Master of Science. Kirov's Agricultural Institute, Faculty of Game Biology.
1982-1986	Post graduate course in All-Union Research Institute of Fisheries and Oceanography, Moscow.
1987	Ph.D. Severtsov's Institute of Evolutionary Morphology and Ecology of Animals, Russian Academy of Science. Moscow.

Professional Experience

2001- present	Alaska SeaLife Center, visiting scientist
1989 – present	Senior Scientist, Chief of Laboratory of Animal Ecology of Kamchatka Institute of Ecology and Nature Management, Far-East Division Russian Academy of Science
1979- 1985	Staff Scientist, All Russian Institute of Fisheries and Oceanography (Moscow), Commander Islands scientific station
1985-1989	Staff Scientist, Chief of Pinnipeds Laboratory of Kamchatka Branch of Pacific Institute of Fisheries and Oceanography
1977-1979	Soviet Army
1977	Inspector, Chukotka Fish Inspection of Okhotskrybvod, Providenie, Magadan region

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Education
Master of Science in Fisheries
University of Alaska Fairbanks 1997

Bachelor of Science in Biology
University of Alaska Southeast 1992

Professional Experience
Research Associate - Alaska SeaLife Center - 6/2000 to present
Primary task – remote monitoring of Steller sea lions

Tour Vessel Captain – Allen Marine, Renown Charters and Tours – 5/1999 to 9/2001
Primary task - Seasonal Captain aboard wildlife tour vessels in Prince William Sound and Kenai Fjords National Park.

Research Technician – National Park Service – 5/1998 to 9/1998
Primary tasks – Monitoring numbers and behaviors of Steller sea lions and Harbor seals in Glacier Bay National Park.

Research Technician – United States Fish and Wildlife Service 5/1992 to 5/1997
Primary tasks – Seabird and marine mammal surveys in Prince William Sound and Lower Cook Inlet.

Additional Assets
USCG Licensed Captain of vessels up to 100 g.t. on near coastal waters.

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Education
Master of Science, Wildlife and Fisheries Science, Texas A&M University, Galveston TX, 2000

Bachelor of Science, Wildlife Science and Resource Management, Virginia Polytechnic Institute and State University, Blacksburg VA, 1997

Professional Experience
Alaska SeaLife Center, June 2001 - Present; Research Associate

Alaska Department of Fish and Game, January – September 2000; Field Technician, Dive Tender

Virginia Polytechnic Institute and State University; 1995-1997; Research Assistant

Virginia Department of Game and Inland Fisheries, November 1995-96; Field Technician

Alaska Department of Fish and Game, June - August 1992; Research Assistant

Alaska Department of Fish and Game, June 1991; Field Technician

Other
Certified SCUBA

AAUS Scientific Diver in training

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Education

B.S.	Animal Science, Reproduction/Embryology	1988 – 1990
	Cornell University, Ithaca, NY	
	Biology, Genetics/Embryology	1986 - 1988
	Cumberland College. Williamsburg, KY.	

Professional Experience

April 2001 – Present
Science Program Coordinator, Alaska SeaLife Center, Seward, AK.
October 1991 – March 2001
Endocrine Research Biotechnician, Smithsonian Institution / National Zoological Park / Conservation and Research Center. Front Royal, VA.
June 1990 - December 1990
Research Technician / Animal Handler, New York State College Of Veterinary Medicine, Cornell University. Dept. of Clinical Sciences and Medicine/ Dept. of Physiology.
December 1989 - June 1990
Technician / Undergraduate Research, New York State College of Veterinary Medicine, Cornell University. Dept. Animal Science/Dept. of Physiology
September 1987 - Will1988
Teaching Assistant / Biology Tutor, Cumberland College. Dept. of Biology Williamsburg, KY
May 1987 - September 1987.
Corbin Animal Clinic. Corbin, KY. Veterinary Technician

Publications

Hosack, D.A., Monfort, S.L., Miller, K.V., **Mashburn, K.L**., Ware, L., and Marchinton, R.L. (1999) Stag exposure advances LH surge and behavioral estrus in Eld's deer (*Cervus eldi thamin*) hinds after CIDR device synchronization of estrus. *Theriogenology* 51(7):133-142.

Garrot, R.A., Monfort, S.L., White, P.J., **Mashburn, K.L**., and Cook, J.G. (1998) One sample pregnancy diagnosis in elk using fecal steroid metabolites. *J. Wildl. Dis.* 34(1):126-131.

Thompson, K.V., **Mashburn, K.L**., Monfort, S.L. (1998) Characterization of estrus cyclicityin the sable antelope (*Hippotragus niger*) through fecal progestagen monitoring. *Gen. Comp. Endo*. 112:129-137.

Monfort, S.L., **Mashburn, K.L**., Brewer, B.A. and Creel, S.R. (1998) Evaluating adrenal activity in African wild dogs (*Lycaon pictus*). *J. Zoo. Wildl. Med.* 29(2):129-133.

Monfort, S.L., Creel, S., **Mashburn, K**, Wasser, S.K. (1997) Steroid metabolism and validation of noninvasive endocrine monitoring in the African wild dog (*Lycaon pictus*). *Zoo Biol*. 16:533-548.

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Education
Doctoral degree in Zoology (s.c.l.), University of Bielefeld, Germany, 1992
Diplom degree in Biology, University of Freiburg, Germany, 1988

Professional Employment
Associate Research Scientist, Texas A&M University at Galveston, TX (1998 - present)
Assistant Research Scientist, Texas A&M University at Galveston, TX (1996 - 98).
Postgraduate Research Physiologist at the Scripps Institution of Oceanography (1992-96).
Doctoral dissertation, Max-Planck-Institute for Behavioral Physiology, Seewiesen, Germany (1990 - 92).
Technician with Dr.G.L.Kooyman at the Scripps Institution of Oceanography, on a research expedition to Antarctica investigating diving behavior of emperor penguins (1989 - 90).
Technician with Dr. G.L. Kooyman at the Scripps Institution of Oceanography, San Diego, CA, on a one-year overwintering research expedition to Antarctica investigating diving behavior of weddell seals (1980 - 82).

Experience
Director & Founder, Laboratory for Applied Biotelemetry & Biotechnology at Texas A&M University Galveston (1999 - present).
Associate Professor of Marine Sciences (Affiliate) , School of Fisheries & Ocean Sciences of the University of Alaska Fairbanks (1999 - present).
Associate Member of the Graduate Faculty of the Wildlife & Fisheries Sciences Department, College of Agriculture & Life Sciences, Texas A&M University College Station (1999 - present).
Consultant to the U.S.Government (Dept. of Commerce, NOAA/NMFS) to review & guide research efforts relating to Steller sea lions in Alaska (1998, 1999).
Member, Physiology Review Panel, Steller Sea Lion Recovery Team Review Workshops (1999).
Associate Editor for Marine Mammal Science (1996 - 1998).
Sole Proprietor of ULTRAMARINE INSTRUMENTS (1996 - present).

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Education

1999 Ph.D. Zoology, University of British Columbia, Vancouver, B.C.
1990 B.S. Biology, with Honors, University of California at Los Angeles, CA

Professional Experience

2002 – present	Assistant Research Professor, University of Alaska Fairbanks and Scientist, Alaska SeaLife Center
1999 – 2001	Postdoctoral Fellow, Zoology Dept., University of British Columbia
1992-99	Research Assistant, Zoology Dept., University of British Columbia
1991-92	Teaching Assistant, Zoology Dept., University of British Columbia

Publications

Darveau, C.-A., Suarez, R.K., **Andrews, R.D**., and Hochachka, P.W. (2002) Allometric cascade: a unifying principle of body mass effects on metabolism. *Nature* (in press).

Enstipp, M. R., **Andrews, R. D**. and Jones, D. R. (2001). The effects of depth on the cardiac and behavioural responses of double-crested cormorants (*Phalacrocorax auritus*) during voluntary diving. *J exp. Biol.* 204: 4081-4092.

Andrews, R. D., Costa, D. P., Le Boeuf, B. J., and Jones, D. R. (2000). Breathing frequencies of northern elephant seals at sea and on land revealed by heart rate spectral analysis. *Resp. Physiol* 123:71-85.

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Andrews, R. D. (1998). Instrumentation for the remote monitoring of physiological and behavioral variables. *J. Appl. Physiol.* 85(5):1974-1981.

Andrews, R. D. (1998). Remotely releasable instruments for monitoring the foraging behaviour of pinnipeds. *Mar. Ecol. Prog. Ser.* 175:289-294.

Webb, P. M., **Andrews, R. D**., Costa, D. P. and Le Boeuf, B. J. (1998). Heart rate and oxygen consumption of northern elephant seals during diving in the laboratory. *Physiol. Zool.* 71:116-125.

Andrews, R. D., Jones, D. R., Williams, J. D., Thorson, P. H., Oliver, G. W., Costa, D. P. and Le Boeuf, B. J. (1997). Heart rates of northern elephant seals diving at sea and resting on the beach. *J. exp. Biol.* 200:2083-2095.

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Education

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Italian Doctorate Universitá degli Studi Department of Biology, Pavia, Italy	Oct 1988

Professional Experience

Research Associate, Alaska SeaLife Center, Alaska	currently
Adjunct Faculty, Biology, Hawaii Pacific University, Hawaii	2000-01
Lecturer, Oceanography, Kapiolani Community College, Hawaii	2000-01
Lecturer, Biology ,Windward Community College, Hawaii	2000-01
Adjunct Faculty, Biology, Transpacific Hawaii College, Hawaii	2000-01
Research Assistant, Hawaii Institute of Marine Biology	1998-00
Teaching Assistant, University of Hawaii at Manoa	1998
Adjunct Faculty, Marine Biology Kansai Gaidai Hawaii College	1997-98
Veterinary Technician VCA Animal Hospital, Hawaii	1997
Research Coordinator, Pacific Cetacean Group, California	1997
Manager, Medical Staff, The Marine Mammal Center, California	1993-94
Veterinary Assistant, Marina Pet Hospital, California	1993
Volunteer Coordinator, Long Marine Laboratories, California	1992
Research Assistant, Tethys Research Institute, Milano, Italy	1992-93
Veterinary Assistant, All Creatures Animal Hospital, California	1992-93
Intern, University of Texas at Austin	1989
Naturalist, Wild Basin, Austin, Texas	1988-89
Naturalist, Austin Nature Center, Austin, Texas	1988-89
Naturalist, Wildlife Rescue Inc., Austin, Texas	1988-89
Research Assistant, Universitá degli Studi di Pavia, Italy	1984-87

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Education

1995 Doctor of Philosophy, University of Alaska, Fairbanks, Alaska

1990 Master's of Science, University of California, Santa Cruz, California.

1987 Bachelor of Science (Honors Marine Biology), University of Guelph, Canada

Professional Experience

Wildlife Biologist III, Marine Mammals Section, Division of Wildlife Conservation, Alaska Department of Fish and Game, AK (August 2000 to present)

Affiliate Assistant Professor of Marine Science, University of Alaska Fairbanks AK (October 2000 – June 2002)

Assistant Professor, Department of Biology, University of Central Florida, FL (1998 to present, currently on leave of absence status)

Research Associate, Institute of Marine Science, University of Alaska Fairbanks, (May-June 1998)

Research Biologist, National Marine Mammal Laboratory, WA (April-May 1998)

Research Associate, National Research Council, tenured at the National Marine Mammal Lab, WA (1995 to 1997)

Graduate Research Assistant, Institute of Marine Science, University of Alaska (1990 to 1995)

Graduate Teaching and Research Assistant, Institute of Marine Sciences, University of California Santa Cruz, CA (1987 to 1990)

Assistant Research Biologist, Tarandus Associates, Toronto, Canada (1987, seasonal)

Assistant Research Biologist, Ontario Hydro Corp., Biological Research Section, Toronto, Canada (1985 and 1986, seasonal)

Robert Eldridge Hicks
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Education
1971 J.D., Harvard Law School
1968 B.A., Stanford University

Diving Appointments
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Instructor, NAUI and PADI, with specialties in cavern, ice, advanced rescue, deep, dry suit, nitrox and wreck penetration

Nitrox Instructor and Blender, IANTD

Scientific Diving Instructor, Alaska SeaLife Center, Alaska Underwater Science Foundation, Prince William Sound Science Center, Alaska Pacific University

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Hyperbaric Chamber Technician, USC Wrigley Institute for Environmental Studies, Catalina Hyperbaric Chamber

Other Professional Experience
Law Clerk, Alaska Supreme Court (1971-1972)
Executive Director, Alaska Judicial Council (1972-1975)
Partner, Hicks Boyd Chandler & Falconer (1975-2001)
Dive Officer, Corporate Affairs Director, Alaska SeaLife Center (2001-present)
Adjunct Instructor, Univ. of Alaska, Anchorage, Alaska, Outdoor and Experiential Education (1996-present)

Publications
2001: "The Jury's In: A Defense Lawyer's Perspective on Risk Management and Crisis Response" in Lessons Learned: A Guide to Accident Prevention and Crisis Response, Deborah Ajango (ed.)

1997: "The Legal Scope of Scientific Diving: An Analysis of the OSHA Exemption" in "Diving for Science, Proceedings of the American Academy of Underwater Sciences."

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Education

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University of Connecticut, Storrs 1994

Master of Science, Marine Biology
University of North Carolina, Wilmington 1989

Bachelor of Science, Marine Biology
University of North Carolina, Wilmington 1986

Professional Experience

1992- Present
Research Scientist, Research and Veterinary Services Department, Mystic Aquarium, Mystic, CT

September 1990-April 1992
Internship, Pinniped Husbandry, Mystic Aquarium, Mystic, CT

July – August 1989
School for Field Studies, Northeastern University, Dolphin Biology and Behavior

Publications

Mazzaro, L.M., Dunn, J.L., Furr, H.C. and Clark, R..M. (1995) Vitamin A kinetics in northern fur seals (*Callorhinus ursinus*) using 3,4-didehydroretinol as a tracer Canadian Journal of Zoology 73:10-14

Mazzaro, L.M., Dunn, J.L., Furr, H.C. and Clark, R..M. (1995) Study of vitamin A supplementation in captive northern fur seals (*Callorhinus ursinus*) and its effect on serum vitamin E Marine Mammal Science 11(4):545-553

Mazzaro, L.M. (1994) Retinol and alpha-tocopherol utilization by captive pinnipeds Dissertation.

Mazzaro, L.M. (1989) A quantitative investigation of cuticular sensory systems associated with the antennular proximal segment of *Callinectes sapidus* Thesis.

Alaska SeaLife Center
windows to the sea

October 23, 2001

To Whom It May Concern;

I am a qualified veterinarian employed full time at the Alaska SeaLife Center and serve on the ASLC Institutional Animal Care and Use Committee.

I hereby certify that the facilities, methods of care and maintenance, and methods of transport proposed for transient juvenile Steller sea lions in this Application will be adequate to ensure the well-being of these marine mammals and will comply with all applicable care and transport standards established under the Animal Welfare Act.

Pam Tuomi, D.V.M.

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EXPIRATION DATE: MAY 15, 2003

USDA

United States Department of Agriculture

Animal and Plant Health Inspection Service

Animal Care

ALASKA SEALIFECENTER

This is to certify that

CLASS R RESEARCH FACILITY

is registered as a

under the

Animal Welfare Act

(7 U.S.C. 2131 et seq.)

Registration No. 96-R-0005

Date of Expiration 1693

Robert McFetridge

Regional Director

Wm. R. Dekban

Acting Deputy Administrator

APHIS FORM 7021 (DEC 96) *Previous editions are obsolete*

United States Department of Agriculture
Animal and Plant Health Inspection Service
Animal Care

INSPECTION REPORT

96-R-0005
96-C-0024

ALASKA SEALIFE CENTER
Name of Licensee/Registrant

Site No.

Licensing No.

8/21 & 22/01
Date of Inspection

Business Name (DBA)
P.O. BOX 1329

Site Name
301 RAILWAY AVENUE

1100 / 1100
Inspection Time

Facility Mailing Address
SEWARD AK 99664
City, State, Zip (for Facility)

Site Address
SEWARD ALASKA 99664
City, State, Zip (for Site)

ROUTINE
Inspection Type

NARRATIVE

NONCOMPLIANT ITEMS NOTED THIS INSPECTION

3.106(a)(3) - WATER QUALITY
WHEN CHLORINE IS ADDED TO THE WATER TO MAINTAIN WATER QUALITY STANDARDS, pH SHALL BE MONITORED AT LEAST DAILY. MONITORING OF pH HAD NOT BEEN ROUTINE DAILY PRACTICE WHEN CHLORINE WAS ADDED TO TANK WATER.

CORRECT UPON SUBSEQUENT ADDITION OF CHLORINE

END OF REPORT

REVISED REPORT

Prepared By: Elizabeth L. Lyons DVM MS
Name & Title: ELIZABETH L. LYONS, USDA, APHIS, Animal Care
VETERINARY MEDICAL OFFICER

Date: 02/05/02

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